IMPACTS OF SHORT- TERM ENVIRONMENTAL MANIPULATIONS ON THE MEAT QUALITY OF THE MUSSEL, *Perna viridis* (Linnaeus, 1758)

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Dedicated to
My
Beloved Parents

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ICAR

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CERTIFICATE

Certified that the dissertation entitled **"IMPACTS OF SHORT TERM ENVIRONMENTAL MANIPULATIONS ON THE MEAT QUALITY OF THE MUSSEL, *Perna viridis* (Linnaeus, 1758)"**, is a record of independent bonafide research work carried out by **Ms. Annie Selva Sonia. G** (MC- 83) during the period of study from September 2002 to August 2004 under our supervision and guidance for the degree of **Master of Fisheries science (Mariculture)** at the Central Marine Fisheries Research Institute, Cochin, and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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DECLARATION

I hereby declare that the dissertation entitled **"IMPACTS OF SHORT- TERM ENVIRONMENTAL MANIPULATIONS ON THE MEAT QUALITY OF THE MUSSEL, *Perna viridis* (Linnaeus, 1758)"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

अल्पकालीन पालन रीतियों के ज़रिए हरित शंबु *पेर्ना इंडिका* के मांस का गुण वर्धन इस शोध पत्र का विषय है. इस केलिए समुद्र जल, निस्यंदित समुद्र जल और धुमानेवाले समुद्रजल की तीन प्रणालियों में हरित शंबुओं का पालन किया. हरित शंबुओं और इनके पलनेवाले पानी में पले बाक्टीरिया - भार का आकलन किया. इन तीनों आकलनों की तुलना प्राकृतिक संस्तरों में उसी अवधि में पाए गए हरित शंबुओं के साथ किया. परिणाम यह निकला कि धुमानेवाले पानी व्यवस्था में बाक्टीरिया की बढती सब से कम होती है, पीछे निस्यंदित व्यवस्था आती है. अतः इस अल्पकालीन उपचार से पानी व शंबु में बसनेवाले *कोलिफार्म* और *स्ट्रप्टोकोक्की* बाक्टीरियाओं को पूरी तरह हटाया जा सकता है. परीक्षणार्थ अवधि में परीक्षणार्थ शंबुओं को संवार्धित सूक्ष्म शैवालों से यथेच्छ खिलाने पर यह भी स्पष्ट हो गया कि इससे मांस का पौष्टिक गुण बर्द्धन साध्य है. इनके मांस में प्रोटीन, कारबोहाइड्रेट का प्रतिशत और मांस सुखाने पर भार बढते हुए देखा. अतः इस प्रकार उल्पकालीन उपचार से शंबु मांस का पौष्टिक गुणवर्द्धन के अतिरिक्त अनुपयोगी बाक्टीरियाओं का दूरीकरण भी साध्य है.

ABSTRACT

Three different set of experiments were conducted using normal seawater, filtered seawater and re-circulatory system for the improvement of meat quality of the green mussel (*Perna viridis*) in terms of bacterial quality and biochemical composition. Total plate count, faecal streptococci count, and coliforms were examined to analyse the bacterial load of mussel and the surrounding water. The most significant bacterial reduction was obtained in the re-circulatory system followed by filtered seawater. Coliforms and faecal streptococci were able to completely eliminated from the mussels through these treatments. The experimental mussels were fed *ad libitum* with cultured micro algae during the experimental period. The nutritional quality of mussel meat studied in terms of biochemical components showed clear significant difference between control and experiment. The condition index, dry meat weight, protein and carbohydrate percentage of the mussel meat was found to increase significantly at the end of all the experiments. The studies showed that the meat quality of the mussels harvested from nature could be improved in terms of bacterial reduction and at the same time increasing the nutritional quality through these types of short-term treatment in captivity by providing feed and quality seawater.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

The fast growing human population demands continuous protein rich quality food items and the attraction of the luxury group towards the seafood exerts continuous pressure on the fisheries resources available for exploitation in the natural water bodies. Marine molluscs like mussels, oysters, clams, scallops, abalones, squids and octopus etc. are attractive delicious seafood items in the internal and international market. These constitute natural resources of sizeable magnitude in many parts of the world from both coastal and inshore areas and available in all ecosystems of marine, brackish and fresh water bodies.

Food security will be a major issue facing mankind in the coming millennium and researchers have turned more seriously to water-based production systems, mainly to meet the increasing demand of food/proteins – deficit to sustain the exploding global population. Marine molluscs are unique for their attributes like rapid growth rate and sturdiness coupled with high tolerance to environmental variations, ease of breeding and good market demand all of which lead to a well established farming systems world over and its culture can supplement the additional requirements in the food supply.

After fish, molluscs are the most commonly cultivated marine organisms in the world. It is believed that the first attempted mariculture was that of molluscs. Japanese farmed oysters on inter tidal stretches of the shore around 2000 B.C. Aristotle mention the cultivation of oysters in Greece while Pliny gave details of Roman oysters farming from 100 B.C. Mussels and oysters represent about 90% of the molluscs that are cultivated. Marine mussels form one of the most dominant cultivable species all around the world. They give the highest conversion of primary producers to human foods and culture of mussels in the water column can increase seafood production several folds with minimum time.

From the nutritional point of view, mussels possess a very high position, as they are high in protein and iron and are very low in calories, fat and cholesterol. When planning a meal with an emphasis on nutrition, mussels can offer balance, based on their nutritional value. According to Food and Drug

Administration's criteria, mussels are extra- lean meat. 100 gram of mussel provides close to entire daily dietary requirement. Mussels are rich in Omega – 3 types of unsaturated fatty acid and the content is higher in it than in any other shellfish. Mussel meat is tender, easily digestible and therefore it could be the cheapest and most nutritious shellfish meat in the world market. Mussels have almost the same protein content per weight of beef meat, but only one quarter of the calories. It is also a source of iron and zinc for immunity booster and mental alertness. Mussels are also used for water purification and for extraction of chemicals and medicines.

With the worldwide popularity of mussels as an edible mollusc, it is not surprise that over 2, 57, 315 metric tonnes of mussels from the wild and 1,370,631 metric tonnes of farmed mussels were landed in 2001 (FAO). India has two commercially important species of mussels, Green mussel, *Perna viridis* and the brown mussel, *Perna indica*. Green mussel has a wide distribution along the Indian coasts and about 10,000 tonnes of mussels out of a potential of 22,080 tonnes are exploited yearly from the west coast of India mainly from the state of Kerala (Appukuttan *et al*., 2001). In India, the total production of mussels from both capture and culture was 15, 066 tonnes in 2002. In India the production from farming was negligible in 1995 but increased to 1, 250 tonnes in 2002 (Modayil, 2003). Commercial mussel culture activity along the south west coast of India picked up in a big way since 1997 in Kerala and Karnataka (Mohamad *et al*., 1998) and cultured mussel production projected to 12, 000 tonnes by 2007-2008. These facts imply increased consumption of the resources. Under these circumstances, it is pertinent to evaluate the quality of mussel in terms of bacterial load and bioaccumulation of heavy metals to develop various techniques to improve the quality for consumption.

Food eating habit of the people is changing very fast particularly in recent times, due to improvement in socio-economic conditions of the people, availability of new resources, application of enriched, prepared foods etc, for the convenience of customers. In this changing situation, nutritive and quality foods have very high demand regardless of its price in the internal and international markets. Seafood is an important item attracting many people of recent times as it is highly delicious and nutritious and it provides a good balance of protein, vitamins and minerals with low calorie. Even after three and half decades of fisheries development

in India, the sea food export industry still depends on one single item namely frozen shrimp, though others like lobsters, squids, cuttlefish and few fishes earn foreign exchange to some extent. In recent years, as bivalves are becoming popular seafood items in many countries in variety of cocktails and modern dishes, diversification in the export sector is an urgent need of the hour in our country.

Shallow waters of the coastline and brackish water areas are the ideal environments for the growth of bivalve molluscs in India. In the same way these areas are often subjected to pollution from different sources. Since molluscan bivalves like clams, mussels and oysters are filter feeders, their meat is likely to contain large quantity of mud, sand, microorganisms and they accumulate large number of bacteria in their body from their environmental water. Generally the profile of the bio-accumulation will be a true reflection of the bacterial profile of their environmental water and the presence or load of these bacteria in the meat is the criteria that adversely affect the consumption and the export of the mussel meat.

The increased recognition that the world is moving rapidly towards global economy with vastly increased international trading of foodstuffs also necessitates the task of ensuring seafood safety (Garrett *et al.* 1997). However a number of problems or obstacles must be solved or minimized to succeed in seafood trade. The success can be achieved by ensuring the safety of seafood; microbiological safety is one of the potential public health issue associated with seafood safety. With globalization, seafood trade will be subjected to increase in greater regulation, control and issues related to environmental practices. Seafood safety would assume greater significance in the future. Eco – labeling and HACCP certification would be made mandatory for all seafood products. Contaminants frequently monitored include bacterial loads, heavy metals, antibiotics and pesticides, algal blooms for HAB (Harmful Algal Bloom) toxins. Quality control agencies for mussel include the Bureau of Indian Standards (BIS), International Organization of Standards (ISO 9002) and European Economic Community (EEC).

In order to develop techniques to decrease the bacterial load of the harvested mussels and at the same to improve the biochemical composition of the meat in captivity experiments were designed and conducted. The aim of this study is by exploiting the filter feeding characteristics of the animals minimize the bacterial contamination using quality sea water and at the same time to improve the meat quality in terms of biochemical composition by giving additional food to the harvested mussels for short periods in captivity. These studies are expected to provide information on the amount of quality manipulations that can be achieved in short periods under captivity for consumer safety and market development. These small scale experiments are preliminary studies aiming to its applications in the field for the short term fattening of harvested mussels in ponds with rich algal blooms and good quality seawater as a final touch or value addition technique to attract the consumers and markets. To achieve the basic concept of consumer preference and safety of the mussels as a food commodity and thus to increase its export potential, the present studies were planned.

The main objectives of the study are

- To analyse the bacterial quality of the harvested mussel meat and the surrounding water body from where these mussels were collected.
- To estimate the biochemical composition of harvested mussel meat.
- To know the effect of short-term captive treatments in terms of bacterial reduction and improvement of nutritional quality.
- Captive treatments tried were rearing the harvested mussels in uncontaminated normal seawater, sand filtered seawater and in re-circulatory system for short duration by providing enough feed during the experimental periods.

Review of Literature

2. REVIEW OF LITERATURE

The commonest of marine molluscs, 'the mussels' are nutritious and delicious. Their sedentary habitat and fast growth rate makes them suitable candidates for cultivation. Due to these qualities they have been extensively used as model organisms in many scientific studies and voluminous data is available from basic environmental, physiological, biochemical, genetic and toxicological investigations as these as sessile filter feeders and have been effective concentrators of environmental contaminants including trace metals (Widdows and Donkin, 1992). There has been a wealth of published information on mussels in the traditional areas of investigations (Korringa, 1952; Kuriakose and Nair, 1976., Kuriakose, 1980; Lutz, 1979; Appukuttan *et al.*, 1980, 2001; Lutz *et al.*, 1980; Silas, 1980; Perez Camacho et al., 1995; Mohamed *et al.*, 1998; Soletchnik *et al.*, 2001; Modayil, 2003) as well as in the applied fields like nutrition, environmental monitoring, population genetics, toxicology, disease and public health (Canzonier, 1988; Kramer *et al.*, 1989; Bonadonna *et al.*, 1990; Kautsky *et al.*, 1990; Dittman and Robles, 1991; Gardner and Skibinski, 1991).

Mussel beds are located in the coastal seas and estuaries of major rivers where heavy loads of different kinds of pollutants are accumulated from the near by lands and thus causes contamination of the water bodies. It is reported that being filter feeders and also bottom feeders bivalves eat all the detritus and dirt and hence, their meat is likely to contain large quantities of mud, sand and microorganisms (Balachandran and Surendran, 1985; Surendran *et al.*, 1986; Nambudiri *et al.*, 1995). Therefore, many investigators studied the distribution of organic contents and microbes in bivalve molluscs (Hatanaka, 1940; Nagabhushanam and Mane, 1975; Stephen, 1980; Ansell *et al.*, 1980, Thangavelu and Sanjeevaraj, 1988). It is further reported that mussels accumulate waterborne contaminants through lower food chain organisms, and are ideal organism for monitoring environmental pollution (Viarengo and Canesi, 1991; Widdows and Donkin 1992).

All these investigators opined that mussels are good representatives of the environment where they grow as they accumulate and deposit all the materials in their body. Therefore many researchers had done extensive studies on the nature of molluscan micro flora (Balachandran and Surendran, 1984, 1985., Surendran *et al.*, 1986). Bacteriological studies on different groups of molluscs showed that both faecal streptococci and coliforms are invariably present in clams while they are in lesser limits in mussels and oysters (Balachandran and Surendran, 1985). However it is reported that mussels are likely to accumulate pathogenic bacteria such as *Salmonella sp., Staphylococcus sp.,* and *Clostridium sp.* (FAO and WHO, 1974). Coliform group of bacteria are known to be a valuable indicators of sanitary quality of finfishes, shellfishes, water and other foods (Hobbs, 1983).

The quality requisites of bivalve molluscs are primarily dependent on the quality of the aquatic environment, assuring a healthy product and safe consumption. Sewage - contaminated bivalve molluscs can cause a significant health risk if consumed raw or lightly cooked (Rippey, 1994; Cliver, 1997; Dore *et al.*, 2003). Investigators pointed out that contamination of mussels, as a food commodity, poses danger to public health (Hackney *et al.*, 1992; Shumway, 1992; Dato-Cajegas and Lin, 1996). The market for bivalves is predominantly for live and/or fresh mussels; therefore the quality of the fresh meat is very important.

With an objective to develop a suitable purification system for bivalves many groups of investigators have developed different purification techniques in different parts of the world. Cleaning of bacteria-contaminated oysters using their own physiological filtration mechanism was developed at the Fisheries Experimental Station, Conway, U. K. (Dodgson, 1928). He found that depuration was an effective method for reducing the microbial flora of contaminated shellfish; this method has been adopted as the best technique for reducing the potential risk of public health hazards associated with the consumption of shellfish which might have accumulated high levels of bacterial or viral pathogens.

Depuration is the process of purification of shellfish in which the animals are placed in disinfected re-circulating or running sea water and allowed to actively filter feed and this process leads to elimination of bacteria from the live

bivalves (Souness *et al.*, 1979; Surendran *et al.*, 1986; Nambudiri *et al.*, 1995). The technology of depuration has been well studied (Huntley and Hammerston, 1971; Neilson *et al.*, 1978; Souness *et al.*, 1979; Aurora *et al.*, 1983), and reviewed (Furfari 1976; Fleet 1978). Where bivalves are sold as live product the treatment process most commonly used is depuration, which represents a major control point during the production of bivalve molluscs worldwide (Richards, 1998). Nagappan Nair *et al.* (1983) studied the needs of purification of farm grown oysters that are supposed to be having better quality than the natural ones and they emphasized that purification by scientific methods in shellfish is vital before they are marketed even if it is cultured.

Mussels generally have higher levels of standard heterotrophic plate count, total coliforms, and faecal coliforms than water because they filter feed on micro-particles including bacterial contaminants and these get accumulated in its body (Dato-Cajegas, 1996). Among the coliforms, in human source 96.4% is faecal coliform and among animal source 93-98% is faecal coliforms (Geldreich, 1978). Faecal coliform bacteria are more directly associated with faecal contamination from warm-blooded animals than total coliforms, and FDA has approved their use as an indicator of faecal pollution in market level shellfish (USFDA, 1984). Faecal streptococci group has been considered as an excellent indicator of human and animal faecal pollution (Holdeman *et al.*, 1976).

Based on the criteria of TC < 70MPN.100ml^{-1} and FC < 14 MPN. 100ml^{-1} the levels set by the National Shellfish Sanitation Program (1993) for areas approved for shellfish harvesting. The European Community had already classified the molluscan shellfish harvesting areas based on the faecal coliforms, not exceed 230 MPN /100 g in the flesh of the harvested product and directed the mode of depuration systems followed (EU Shellfish Hygiene Directive 91/492/EEC). Thus, in order to meet safety standards internally and abroad, fisheries and public health officials should institute measures to ensure adequate sanitary quality of shellfish. Such action is paramount to the development of viable and profitable shellfish industry.

The quality requisites for seafood especially the bivalves primarily points to the microbial purity; however, from a nutritional standpoint, other biochemical characteristics also influence the product quality (Beninger and Lucas, 1984; Karakoltsidis *et al.*, 1995). Water, protein, lipid, mineral and glycogen contents of the meat, together with minor components of a hydrophilic nature, contribute to the nutritional value and organoleptic characteristics of mussels. It is known that salinity, water temperature, food availability and reproductive cycle of animals may influence the meat yield and biochemical composition of mussels (Fernandez-Reiriz et al., 1996; Okumus and Stirling, 1998).

Biochemical composition of oyster meat varies with environment, season and physiological condition of oyster (Gabbott, 1983; Martinez, 1991; Stirling and Okumus, 1995; Danovara and Fabiano, 1997). There are studies which shown that the water level in the bodies of bivalves tends to increase or decrease with the changes in salinity of the seawater (Galtsoff, 1964; Ansell *et al.*, 1973; Nagabhushanam and Mane, 1978; Rao *et al.*, 1987). Fluctuation in the moisture content due to absorption of water and loss of solids from the body of animals are the most significant features that cause changes in the chemical composition of the oyster meat (Thangavelu and Sanjeevaraj, 1988). These changes in meat during certain periods lower their commercial value.

A rational and profitable harvesting of oyster lies on the basis of obtaining the highest meat weight with the best biochemical constituents (Thangavelu, 1983). As the nutritional and energy demands of marine animals are not constant, and are affected by exogenous factors such as food availability and temperature and endogenous factors such as energy demands for reproduction, metabolic reserves accumulated in tissues may be used in energy production or converted into various biochemical components (Gabbott, 1983; Martinez, 1991).

Biochemical studies on bivalves have drawn the attention of several researchers because these are major sources of protein, carbohydrate and lipid and have high calorific value (Joshi *et al.*, 1979; Jayabal and Kalyani, 1986; Knauer *et al.*, 1994 and Rivonker and Parulekar, 1995). Jeng *et al.* (1979) studied the chemical composition of Taiwanese oysters and clams and Knauer *et al.* (1994)

studied the proximate composition of the South African abalone, *Haliotis midae*. Many investigators studied the biochemical composition of bivalves in India (Venkataraman and Chari, 1951; Durve and Bal, 1961; Kasinathan 1964 a, b,'67; Joshi and Bal, 1965; Rahaman, 1965; Saraswathy and Nair, 1969; Nagabhushanam and Deshmukh, 1974; George and Nair, 1975; Salih, 1979; John, 1980; Lakshmanan and Nambisan, 1980; Jayabal and Kalyani, 1986).

Apart from these, Ansell *et al.* (1973) studied the biochemical composition of four invertebrates including *Donax* sp. taken from sandy beaches of Cochin and Shertallai. Jayabal (1964) studied the biochemical contents in the estuarine clam *Katelysia opima* from Vellar Estuary. Rivonker and Parulekar (1995) studied the proximate and biochemical compositions and calorific potential of raft cultured green mussel *Perna viridis*. Other works on biochemical composition of bivalves in Indian are on *Katelysia marmorata* (Joshi and Bal, 1965), *Meretrix casta* (Salih, 1979; Balasubrahmanyan and Natarajan, 1980), *Villorita cyprinoids* (Ansari *et al.*, 1981) and *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977; Victor, 1984). At the same time, Nagabhushanam and Dheshmukh (1974) studied the seasonal changes in chemical composition of the estuarine clam *Meretrix meretrix*. All these studies revealed that, from the nutritional point of view, the molluscs form one of the best sources of protein, fat and minerals.

Several workers have worked on variation in the nutritive value of various molluscs in different seasons and reported different values according to the season, food availability and reproductive condition (Baird, 1958, 1966; De Zwaan and Zandee, 1972; Dare and Edwards, 1975; Pieters *et al.*, 1979; Lakshmanan and Nambisan, 1980; Zandee *et al.*, 1980; Small and Van Stralen, 1990; Chinnamma George and Gopakumar, 1995). Seasonal changes in meat weight and biochemical composition have been reported in the black clam *Villorita cyprinoids* (Ansari *et al.*, 1981). These studies emphasize that the nutritional value of the bivalves are mainly controlled by factors like salinity, temperature and food availability, which vary according to seasons. High intensity of feeding results in the storage of glycogen and fat content in oysters (Thangavelu, 1983). It is said that mussels are best to be consumed in late autumn and winter, probably due to high nutritive value in these months.

Mussels are more delicious in the pre-spawning conditions and the harvests of mussels are conducted during this phase due to high market demand. Once the reproductive maturity is attained, use of the assimilated material for somatic or reproductive growth will vary according to environmental conditions and food availability (Dare and Edwards, 1975; George and Nair, 1975; Gabbott, 1983; Fernandez Reiriz *et al.*, 1996; Danovara and Fabiano, 1997). Carbohydrate of bivalves comprised mainly glycogen (Gabbott and Bayne, 1973) and the changes in the carbohydrate may be due to accumulation and utilization of glycogen at different stages of gametogenesis and spawning. Humphrey (1941) observed that glycogen value dropped during the spawning period and hence the wet weight of the oyster also declined.

Different parameters used to assess the nutritional quality of mussel meat include condition index, moisture content, dry meat weight, ash content, protein, carbohydrate, lipid etc. A parameter of eco-physiological and economic relevance, especially in view of the industrial processing, is represented by the condition index, a measure of the apparent health and commercial quality of bivalves (Orban *et al.*, 2002). Condition index can be considered as a measure of "fatness" and "marketability" (Baird, 1958). In aquaculture, the condition indices can be used to designate the quality of the product to be marketed (Durve, 1964; Okumus and Stirling, 1998).

Numerous workers have demonstrated that the condition index of various species of bivalves were related to the level of available food and the annual reproductive cycle (Baird, 1958, 1966; Walne, 1970; Westly, 1970; Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Gabbott and Stephenson, 1974; Dare , 1976; Roger Mann, 1979). The dry weights, glycogen estimation, total chemical analysis etc. are the various other parameters widely adopted for assessing the quality of meat (Ansell *et al.*, 1980; Durve, 1964). Several workers studied the changes in body weight, body component index; percentage of water in the body in relation to environment and physiological conditions of bivalves (Venkataraman and Chari, 1951; Durve and Bal, 1961; Durve, 1964; Joshi and Bal, 1965; Nagabhushanam and Mane, 1975, 1978).

Lipids represent an important energy reserve because of their high calorie contents; they are mainly used in chronic stress conditions, whereas glycogen reserves are generally used during gametogenetic processes when lipids are not available. Proteins, the most abundant biochemical component in tissues, may be subjected to metabolic transformation, although they do not undergo such high accumulation processes as lipids and carbohydrates (Gabbott, 1975). *Mytilus edulis* from North European coasts has its highest glycogen contents when abundant planktonic food is available (De Zwaan and Zandee, 1972; Rodhouse *et al.*, 1984). It is likely that seasonal variations, feeding habits, availability of food, and temperature of the habitat and stages of sexual growth would have largely contributed to the vast differences in the biochemical composition.

Claires (oyster ponds) are traditionally used for fattening and greening of oysters. This fattening process allow the oysters to acquire reserve glycogen and lipids which give good taste to the meat and thus possible to improve the meat quality. To optimize the fattening process, large phyto-planktonic blooms were induced in ponds, and then distributed the oysters' ponds after harvesting from the farms. This fattening period is designed to perfect the oyster taste, appearance and colour (Soletchnik *et al.*, 2001). The greening of the oysters in the claires resulted in improvement of the proximate and biochemical composition of the *Crassostrea gigas*. Fattening process allowed the oysters to acquire the taste and often a green colour specific for this rearing area (Soletchnik *et al.*, 2001). Most of the shellfishes harvested locally are in fact harvested from coastal and estuarine waters with varying degrees of pollution caused by domestic sewage discharges. Fattening in good quality seawater with adequate food for a definite short period can purify the harvested mussels as well as improve the nutritional value of the product thus can make the item ideal for consumption and attract the markets.

Material and Methods

3. MATERIAL AND METHODS

Live green mussels *Perna viridis* were collected from natural beds off Narakkal by hand picking and brought to the hatchery in wet gunny bags. The mussel beds located about 2 kms away from the coastline is an area of commercial mussel fishery during every year. Care was taken to ensure that the mussels were uniform in size and free from fouling organisms.

3.1 EXPERIMENTAL DESIGN

Mussels were washed thoroughly in seawater and kept in a basin of seawater, a day prior to start experiment for conditioning. Experimental tanks of 50 liters capacity were filled with seawater, of salinity 29 ± 1 ppt. After measuring the individual length and weight of the mussels, they were transferred to the experimental tanks, provided with good aeration.

Each experiment was conducted for a period of 10 days.The temperature of the water in the experimental tanks was examined daily. Everyday morning before feeding 50% of the water from the experimental tanks was replaced with fresh seawater to maintain the water quality. The faecal matters of the mussels were also removed while exchanging the water from the tanks without disturbing the animals. Salinity of the experimental tanks maintained at 29 ± 1 ppt was adjusted by diluting ambient fresh seawater with dechlorinated tap water and measured using a salinometer. Tanks were checked frequently for mussel mortality.

For feeding the animals, algal cultures were maintained in the laboratory in different phases throughout the experimental period. These mussels were fed *ad libitum* with mixed algae twice in a day. Fifty percent of the feed was given at 9.30 hrs and the remaining at 16.00 hrs to prevent pseudofaeces formation and wastage of feed. Algal cultures were harvested in the exponential phase of its growth. Different species of mixed micro algae developed for feeding *Perna viridis* were *Chaetocerous calcitrans*, *Chlorella salina*, and *Nanochloropsis* sp.

3 sets of experiment were conducted as following:

1. ***Normal seawater system*** :- In which the recently collected, settled, stored seawater was used to replenish the tanks daily.

2. ***Filtered seawater system*** :- The previously collected seawater was pumped to an overhead tank and allowed to filter through a biological filter, set in a bin before adding to the experimental tanks with mussels.

3. ***Re-circulatory system*** :- In which a constant flow through current of filtered seawater system was maintained in the experimental tanks. The water from the experimental tanks was allowed to be collected in a bin (biological filter) by gravitation and from there the filtered seawater was again drawn to the experimental tank by suction force created by a high power aerator.

4. ***Control*** :- For control, mussels kept in cages were tied in a raft and maintained in the open sea (about 2 kms away from the shore) from where the mussels were collected for experiments and used.

Fig.1. Collection site of *Perna viridis*

Fig.2. Microalgal cultures

Fig.3. Filtration unit - Filtered seawater system

Fig.4. Experimental set up - Re-circulatory system

3.2 SAMPLING

Samples were taken periodically for assessing the bacterial quality and also for the analysis of biochemical composition. Sampling was done before the water exchange to confirm the bacteriological load in mussel meat and water. During the experiments, water samples were taken aseptically in 200 ml sterile bottles and mussels were collected in sterile containers and brought to the laboratory for microbiological analyses. Mussels were thoroughly washed in fresh seawater, to remove the adhering dirt's and then the shells were cut open using sterile stainless steel scalpel. Whole body was taken out and wiped well with sterile tissue paper to remove the excess moisture. The wet weights of the mussel tissue were taken before the analyses and the same was used for further analysis.

3.3 ALGAL CULTURE

Stock cultures of mixed micro algae kept in 2 liter Hafkin culture flasks were maintained in air-conditioned room. Autoclaved / boiled seawater after cooling was poured into the Hafkin flasks and required amount of nutrients were added. About 10 ml of the inoculum in the growing phase was transferred to the culture flasks and it was placed in front of 2 fluorescent lights (1000 lux). After 8-10 days, when the maximum exponential phase has reached, light was reduced to 500 lux for further growth. At the time of maximum exponential phase of growth, the colour of the culture turns into dark brown in the case of *Chaetocerous calcitrans*, and green in the case of *Chlorella salina*, and *Nanochloropsis* sp. These stock cultures were used as inoculum for mass culture.

For the mass culture, pre- sterilized transparent plastic buckets of 10-liter capacity were used. Appropriate concentrations of nutrient media were added to these buckets like Walne medium (Walne, 1974). (1 ml / lit A* and B* solution and 0.5 ml silicate / lit). Seawater in each bucket was adjusted up to 6 liters; inoculated with 10% of fully-grown culture from the stock and placed in natural sunlight provided with good aeration. After attaining the exponential phase, the culture was harvested and supplied to the experimental animals in two equal rations.

Solution A*

Potassium nitrate	: 75 g
Potassium orthophosphate	: 5 g
DW	: 1 liter

Solution B*

EDTA	: 4.36g
$FeCl_3$	: 3.15 g
Trace metals	: 1 ml
DW	: 1 liter

3.4 EXPERIMENTAL PROCEDURES

3.4.1. Bacterial quality of mussel meat and water

3.4.1.1 Preparation of sample

Aseptically mussels were cut open with a sterile stainless steel scalpel and the meat was transferred into a sterile petri dish using sterile scissors for the analysis of bacterial quality. For analyzing the bacterial quality of mussel meat, ten grams of the meat sample was aseptically weighed into a sterile sample dish. The sample was transferred into a mortar and macerated with pestle (sterilized by burning with alcohol and flaming). 90 ml sterile normal saline (0.85%) was added into the mortar and mixed uniformly. This becomes 10^{-1} dilution of sample. Using a sterile 1 ml pipette, 1 ml of supernatant was aseptically transferred into a 9 ml sterile normal saline tube and the contents were thoroughly mixed using Vortex mixer. This becomes 10^{-2} dilution. Similarly, further serial dilutions were prepared up to 10^{-6} and this was used for the enumeration.

For analyzing the bacterial load in water sample, 100 ml of water sample was taken in a sterile conical flask. Using a sterile pipette, 1 ml of water

sample was aseptically transferred into a 9 ml sterile saline tube. This becomes 10 times dilution and similarly further dilutions were made up to 10^{-6} and used for the enumeration.

3.4.1.2. Total Plate Counts (TPC)

Carefully 1 ml each of appropriate dilutions was pipetted to appropriately marked sterile Petri dishes taken in duplicates for each dilution. About 15-18 ml of molten Zobell Marine Agar (ZMA) was cooled to 45°C and poured to each plate. The plates were mixed well with the inoculum and allowed to set for 30 minutes. The set plates were incubated at 37° C for 48 hours in an inverted position. After the incubation, the individual bacterial colonies developed in each plate were counted using Quebec colony counter. The average count of the duplicates was also calculated. The Total Plate Count (TPC) per gram of the sample was calculated as follows:

TPC / g of the sample = (Average count x dilution factor)/ Weight of the sample (g)

3.4.1.3. Faecal streptococci count

In order to study the presence of faecal streptococci, appropriate dilutions (0.5 ml each dilutions) were pipetted into 1 ml TTC /100ml added, pre-set and dried Kenner Faecal Agar plates, taken in duplicates for each dilution. The inoculum was spread well over the surface using a sterile bent glass rod. The plates were incubated at 37°C in an inverted position for 36 - 48 hrs. All the surface and subsurface light pink to deep red colonies were counted as Faecal Streptococci. The average of the duplicates was taken and the Faecal Streptococci count per gram sample was calculated as follows:

Faecal Streptococci count / gram sample = (Average count x 2 x dilution factor)/ sample weight.

3.4.1.4 Total coliforms and Faecal coliforms

The coliform group of organisms was determined by the multi-tube test includes all aerobic and facultative anaerobic gram negative non-spore forming rods that ferment lactose with gas formation at 48 hrs at 35°C. For meat and water sample, Most Probable Number (MPN) method of enumerations were conducted using 3 and 5 tube dilutions respectively with lauryl sulphate tryptose or lactose broth in which growth and gas production is observed for positive results.

A) 3-tube method followed for mussel meat sample

This method included the following 3 steps:

Step: 1 (for presumptive total coliforms)

From the homogenate (10 g sample + 90 ml normal saline) of sample, 10 ml was inoculated in duplicate to the 3 tubes of DS MC broth, 1 ml each in duplicate to 3 tubes of SS MC broth and 0.1 ml each to the next 3 tubes of SS MC broth and labeled appropriately. Then the tubes were incubated in the serological water bath at 37°C for 24 hrs.

After 24 hrs the tubes were observed for growth and gas production. MC broth in tubes that turned towards yellow with gas production in Durham's tubes was considered as positive tubes. Number of positive tubes in each set of 10 ml, 1 ml and 0.1 ml tubes were noted and compared with Standard 3-tube MPN table to get MPN values for presumptive total coliforms count.

Step 2: (for confirmed total coliforms)

From the positive tubes of step 1, one loopful of culture was taken using a sterile inoculation loop and inoculated to BGLB 2% broth and marked the corresponding label. The tubes were then incubated at 44.5 ± 0.5°C in a serological water bath for 24 hrs. After 24 hrs the tubes were observed. Tubes with growth and

gas production in Durham's tubes were considered as positive tubes. The results were compared with Standard 3-tube MPN table to obtain the MPN values for confirmed total coliforms.

Step 3: (for faecal coliforms)

From the positive tubes of step 2, one loopful of culture was taken using a sterile inoculation loop and inoculated to EC broth and labeled appropriately. The tubes were incubated at 44.5 ± 0.5°C in a serological water bath for 24 hrs. After 24 hrs the tubes were observed for growth and gas production in Durham's tubes as positive tubes. The number of EC broth positive turbid and gas producing tubes in each set was compared with the Standard MPN table to get the MPN values for faecal coliforms.

B) 5-tube method followed for water sample

This method included the following 3 steps:

Step: 1 (for presumptive total coliforms)

50 ml of the water sample was inoculated into 50 ml DS MC broth in duplicates, 10 ml of water sample was inoculated into each 10 ml DS MC broth in duplicates and 1 ml of sample in to each 10 ml SS MC broth in duplicates was inoculated and labeled appropriately. Then the tubes were incubated in the serological water bath at 37°C for 24hrs.

After 24 hrs the tubes were observed for growth and gas production. MC broth in tubes, which were, turned towards yellow with gas production in Durham's tubes were considered as positive tubes. Number of positive tubes in each set of 10 ml, 1 ml and 0.1 ml tubes were noted. Results were taken by comparing with standard 5-tube MPN table and the values obtained for presumptive coliforms were noted.

Step 2: (for confirmed total coliforms)

From the positive tubes of step 1, one loopful of culture was taken using a sterile inoculation loop and inoculated to BGLB 2% broth and marked the corresponding label. The tubes were then incubated at 44.5 ± 0.5°C in a serological water bath for 24 hrs. After 24 hrs the tubes were observed for taking result. Tubes with growth and gas production in Durham's tubes were considered as positive tubes. Results were then compared with Standard 5-tube MPN table to obtain the values for confirmed total coliforms.

Step 3: (for faecal coliforms)

From the positive tubes of step 2, one loop full of culture was taken using a sterile inoculation loop and inoculated to EC broth and labeled appropriately. The tubes were incubated at 44.5 ± 0.5°C in a serological water bath for 24 hrs. After 24 hrs the tubes were observed for growth and gas production in Durham's tubes as positive tubes. Then the number of EC broth positive turbid and gas producing tubes in each set were compared with the Standard 5-tube MPN table to get the MPN values for faecal coliforms.

3.4.1.5 Media used

The media and chemicals used for microbiological study were the supply of Hi Media Laboratories Ltd., Mumbai.

The list of media / broth and solution used are given below

A) Normal saline / Physiological saline

Nacl	:	0.85 g
DW	:	100 ml

Salt weighed and dissolved in 100 ml distilled water. Distributed in appropriate quantities (95 ml or 9.5 ml). Sterilized at 15 lbs for 15 min.

B) Zobell Marine Agar

Peptic digest of animal tissue:		5 .0 g
Yeast extract	:	1 .0 g
Ferric citrate	:	0. 1 g
Sodium chloride	:	19.45 g
Magnesium chloride	:	8.8 g
Sodium sulphate	:	3.24 g
Calcium chloride	:	1.8 g
Potassium chloride	:	0.55 g
DW	:	1 lit.

All the ingredients were boiled to dissolve completely and Sterilized by autoclaving at 15 lbs for 15 minutes.

C) Kenner Faecal Agar (KFA)

Peptone	:	1.0 g
Yeast extract	:	1.0 g
NaCl	:	0.5 g
Sodium glycerophosphate:		10 g
Maltose	:	2.0 g
Lactose	:	0.1 g
Sodium azide	:	0.04 g
Bromocresol purple (BCP):		0. 0015 g
Agar	:	1.5 g
DW	:	100 ml
pH	:	7.2 ± 0.1

Except BCP all other ingredients were weighed and dissolved in distilled water, pH adjusted for 7.2 ± 0.1, 1.5 ml of 0.1% BCP solution was added. Sterilized at 10 lbs for 20 mins. Before pouring into plates, 1 ml of 1% Triphenyl Tetrazolium Chloride (TTC) was added to the molten medium cooled to 45°C.

D) TTC solution

Triphenyl Tetrazolium Chloride	:	1 g
DW	:	100 ml

Dissolve 1g Triphenyl Tetrazolium Chloride in 100 ml sterilized distilled water by steaming for 1 hr.

E) Modified MacConky Broth (For MPN)

(Double strength and single strength)

Peptone	:	2.0 g
Lactose	:	1.0 g
Bile salt (No.3)	:	0.5 g
NaCl	:	0. 5 g
Bromocresol purple (BCP)	:	0.001 g
Crystal violet	:	0.0001 g
Distilled water	:	100 ml
pH	:	7.4 ± 0.1

All the ingredients except the two dyes were weighed for 200 ml and dissolved in 100 ml distilled water. pH was adjusted to 7.4 and 1 ml of 0.1% BCP and 0.05 ml of 0.2% Crystal violet solutions were added. 50 ml portion of the medium was distributed in 10 ml quantities into 5 large test tubes (150 mm x 25 mm) and the rest of the medium was diluted to 100ml by adding 50 ml distilled water to make single strength medium. Dispensed in 10 ml quantities into 150 mm x 18 mm dia test tubes with inverted Durham's tubes. Plugged with nonabsorbent cotton and sterilized at 10 lbs for 20 min.

F) Brilliant green bile broth 2% (BLGB)

Peptone	:	1.0 g
Lactose	:	1.0 g
Bile salt	:	2.0 g
Brilliant green	:	0. 00133 g
DW	:	100 ml
pH	:	7.4

All ingredients were dissolved; pH adjusted and dispensed in 5 ml quantities in 100 mm x 12 mm tubes (with inverted Durham's tubes). Sterilized at 10 lbs for 20 min.

G) *Escherichia coli* Broth (EC broth)

Tryptone	:	2.0 g
Lactose	:	0. 5 g
Bile salt (No.3)	:	0.15 g
KH_2PO4	:	0.15 g
K_2HPO4	:	0. 4 g
NaCl	:	0. 5 g
DW	:	100 ml
pH	:	6.9 ± 0.1

All ingredients were dissolved; pH adjusted to 6.9 ± 0.1 and dispensed in 5 ml quantities in 100 mm x 12 mm tubes (with inverted Durham's tube). Sterilized at 10 lbs for 20 min.

3.4.2 Condition index of animals

Condition index of the mussels was taken during each sampling to know the plumpness of the meat. Care should be taken to ensure that it is free from dirt or other fouling organisms on the shell. The total weight of mussel meat dried at

80°C overnight in a hot air oven and weighed in a tarred vessel using a Mettler analytical balance. Similarly the shells were dried at 100°C for 24 hours, cooled under desiccation and weighed. The condition index was calculated using the following formula (Walne and Mann, 1975).

$$\text{Condition index} = \frac{\text{Dry meat weight (g)} \times 1000}{\text{Dry shell weight (g)}}$$

3.4.3 BIOCHEMICAL COMPOSITION OF MUSSEL MEAT

3.4.3.1 Moisture content

The tissue samples were cleaned and the water adhering to them was removed using a blotting paper. The wet weight of the tissues were taken accurately and the samples were gradually dehydrated to constant weight in a hot air oven at 100 ± 2°C for about 16 hrs. Then the samples were taken out, cooled in a desiccator and again weighed. The moisture content was then calculated gravimetrically as the difference in the wet weight and the dry weight of the tissue and was expressed as percentage of wet weight. The estimation was done in duplicate.

$$\text{Moisture content (\%)} = \frac{\text{(Wt. of fresh sample} - \text{Wt. of dry sample)} \times 100}{\text{Wt. of fresh sample}}$$

3.4.3.2 Dry meat weight

Dry meat weight was calculated using the given formula,

$$\text{Dry meat (\%)} = \frac{\text{Wt. of dry sample} \times 100}{\text{Wt. of sample before drying}}$$

After determining the moisture content, the samples were dried at 100 ± 2°C in hot air oven. Dried samples were powdered in a mortar, transferred into labeled stoppered glass vials and stored in desiccators for further analysis. For all the biochemical estimations extra pure or 'Anala R' grade chemicals were used.

3. 4.3.3 Ash content

A pre-weighed amount of oven dried powdered tissue sample was ignited in a tarred silica crucible for 5 hrs at 600°C in a muffle furnace, till all the organic matter was burnt out leaving no carbon residue. The ignited content was weighed after cooled to room temperature and the difference in the weight taken as the ash content of the tissue.

The percentage of the ash content of the tissue was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{Ash weight} \times 100}{\text{Dry weight of tissue}}$$

3.4.3.4 Protein

Accurately 0.5 gm of dried powdered samples was taken in to clean and dry digestion tubes, and the exact weights were noted and the tubes were labeled appropriately. Approximately 1 g of digestion mixture (K_2SO_4 + $CuSO_4$ in the ratio 9:1) was added and mixed thoroughly, then 5 ml of nitrogen free concentrated sulfuric acid was added to each tube and the flasks were heated gently at an inclined angle until frothing subsides and then boiled until the solution appears green colour. Cooled the tubes to room temperature and the contents were carefully transferred in to a 100 ml volumetric flask. Tubes were repeatedly washed with ammonia free distilled water and the washings were collected in to the volumetric flask. Volume was made up to the mark. Micro kjeldahl distillation assembly was started and after the steam generated, receiver flask was washed 3 times with double distilled water using the negative pressure created by cooling.

5 ml of the sample aliquot was taken, in Micro kjeldahl distillation unit, followed by 40 % NaOH till colour is formed. Steam was allowed to pass through the flask which converts ammonium sulphate to ammonia and was collected in a 10 ml of 4 % boric acid solution containing mixed indicator (Methyl red and Bromocresol green) placed in clean conical flask. The time of distillation was approximately 5 - 10 minuets. Back titrated the green coloured boric acid solution with 0.1 N HCl in burette, the end point changed to pink and the volume of acid consumed (V) was noted, repeated the experiment for concordant titer values. The percentage of crude protein was calculated as follows:

$$\text{Crude Protein (\%)} = \frac{\text{Volume of HCl in ml (V)} \times 0.0014 \times 100 \times 100 \times 6.25}{\text{Weight of sample (W)} \times 10}$$

3.4.3.5. Carbohydrate

The phenol sulphuric acid method of Dubois *et al.*, (1956) was followed to estimate the carbohydrate in the mussel samples.

Dry tissue samples, each weighing 10 mg was thoroughly homogenized with 2 ml deproteinising agent (10% TCA) by keeping the tubes in ice. All the samples were centrifuged for 20 min at 3,000 rpm. The supernatant obtained in the individual tubes was used for the estimation of total carbohydrates. From the above supernatant, 0.1 ml was taken and made up to 1 ml with saturated Benzoic acid in double distilled water and to this solution; 1 ml of 5% phenol solution was added. 5 ml of concentrated sulphuric acid was added rapidly and carefully to each tube and mixed well using a cyclomixer.

A standard solution was prepared using D glucose (Conc: 20 mg/100 ml saturated solution of benzoic acid). Different dilutions of the working solution with the concentration of glucose ranging from 10 -100µg/ml were prepared and the procedure adopted for the tissue was followed. A blank solution with 2 ml 5% phenol was prepared and the above procedure followed. All the tubes were kept for 30 min

at 30°C and the OD of the orange colour developed was measured at a wavelength of 490 nm using a Thermospectronic (Genesys 10UV) spectrophotometer with the samples taken in silica cuvettes.

Standard graphs were plotted with the concentration in different dilutions of the working standard solution in the X - axis and OD in the Y - axis. Comparing the OD obtained for the sample with the values in the standard graph and also using the formula, total carbohydrate was calculated.

$$\text{Carbohydrate (\%)} = \frac{\text{OD of the sample x Con. of Std. x 100}}{\text{OD of the Std. x wt. of sample in mg}}$$

3.4.3.6 Total Lipids

Bligh and Dyer (1959) method was followed for the estimation of total lipids in mussel.

5g of dried powdered mussel whole body were taken for total lipid analysis. Distilled water was added to make the moisture content 80% and made it into paste. Chloroform methanol mixture was added (15 times) and mixed ($1/3^{rd}$ of the total volume). Filtered the solution using Whatman filter paper (No. 41) and the filtrate was collected. Repeated the procedure, two more times with rest of the chloroform methanol mixture. To the filtrate, distilled water was added (20% of the total volume of the filtrate) and transferred the content to a 200 ml separating funnel, allowed to stand overnight.

The water-soluble residue diffuses away from the solvent and occupies the top position in the separating funnel. Solvent containing lipid (bottom layer) was collected in a beaker by filtering through anhydrous sodium sulphate. Evaporated the chloroform under reduced pressure using a rotary evaporator and the total lipid was weighed and the percentage of total lipid was calculated as follows:

$$\text{Total lipids (\%)} = \frac{\text{Wt. of lipid x 100}}{\text{Wt. of sample}}$$

3.4.4 STATISTICAL ANALYSIS

For all biochemical parameters, the difference between the control and different set of experiments were tested using Analysis of Variance (ANOVA) using the computer software SYSTAT.

Results

4. RESULTS

The animals for present studies were collected from the coastal waters from where the commercial exploitation of the mussel is done for marketing. The animals collected were 40 - 50 mm in total length. The average total weight and wet meat weight was 8 -10 gm and 1.5 - 3 gm respectively. Samples were taken for the examination of biological details like condition index (CI), maturity stages, bacterial flora, and biochemical composition. Biochemical composition of raw mussel meat, its microbial flora and the bacterial quality of the water from harvest sites were studied to find out the nutritional quality of these mussels during its harvest. Different experiments were designed and conducted to improve the nutritional values to a limit that attracts the markets in much better way and the results obtained are presented. The results of the present investigations revealed that the experiments conducted could improve the biochemical contents of the mussel meat and at the same time it reduces the bacterial counts to permissible limits.

4.1 BACTERIAL QUALITY OF RAW MUSSEL MEAT AND WATER

4.1.1 Total Plate Counts (TPC)

Bacterial counts and the biochemical composition of the mussels from the commercial fishery sites were taken to assess the quality of the natural mussels on harvest and to estimate the amount of quality improvements added due to the short term treatments before its marketing. During the experimental tenure the bacterial counts were analyzed in the control and in all sets of experiment in fixed periods. Samples were taken and various analyses were conducted on the 3rd, 5th, 7th and 10th days of the experiments. The average 'Total plate count' estimated in the control was 37.38 x 10^3 cfu /g on the 1st day (Table 1). Subsequent observations revealed an increase in the total plate count up to 5th day and a decreasing trend in the following days till the end of the experiment. The total plate count attained a value of 42.58 x 10^3 cfu /g on the 10th day of the experiment.

In the experiments with normal sea water systems, the total plate count showed a gradual decrease in the mussel tissues. The average total plate count was 29.13 x 10^3 cfu /g on the 1st day and reached 12.96 x 10^3 cfu /g at the end of the experiment on the 10th day. In the experiments with filtered seawater, the reduction in the total plate count was more remarkable. At the beginning of the experiment TPC was 33.95 x 10^3 cfu /g and at the termination of the experiments on 10th day it reduced much and reached 2.48x 10^3 cfu /g. The total plate count of the mussels in the experiments with re-circulatory water systems was 32.2 x 10^3 cfu /g on the 1st day. A great reduction in bacterial load observed in this experiment as in the case of the 2nd set of experiments with filtered seawater. The reduction in TPC was found sharp and steady from the 3rd day and this continued in the subsequent days till the end of the experiments. The total plate count at the termination of the experiment was only 0.68 x 10^3 cfu /g.

The total bacterial counts in the water where the mussels are retained were also analyzed during the study. In the control the total bacterial count of the surrounding seawater was found consistently lower than the corresponding bacterial counts of the mussels because they filter feed on micro-particles including bacterial contaminants from the surrounding water where they live.

In the experiments with normal seawater the higher TPC occurred in the water on the 3rd day onwards than in the mussels (Table 2). However the higher bacterial counts occurred on the 3rd day, it was found consistently decreased in the subsequent days. From the studies it is found that the average total plate count in the water sample of control on the 1st day was 25.58 x 10^3 cfu/ml and it was found increased in the 5th day to 31.5 x 10^3 cfu/ml. Afterwards a decrease in TPC was observed on the 7th day onwards and the bacterial count on the 10th day was only 24.55 x 10^3 cfu/ml. From the investigations it is found that the TPC in the water as well as in the mussels in the nature is not following a specific pattern but it is varying with the environmental factors especially the contamination of water and the tidal effects which is the leading force that cleanup the coastal waters.

In the experiments with normal seawater system it was observed that the TPC of experimental water in the 1st day was almost equal to the control. However a gradual but steady reduction in the TPC observed and it continued till the termination of the experiment. It was found decreasing through the 3rd, 5th and 7th days and at the end of the experiment it was about 18.85 x 10^3 cfu/ml. With the normal settled fresh seawater systems itself the total bacterial count in the water was found decreased, as there is no addition of new contaminants. Here no additional effort or energy has paid except 50% of the water in the tanks renewed daily and supplied enough feed to the animals.

Whereas in the second set of experiments where filtered seawater systems were used the reduction in the bacterial counts was sharp and rapid. The TPC on the 1st day in the filtered seawater was 17.5 x 10^3 cfu/ml and the same reached 4.6 x 10^3 cfu/ml on the 10th day. The total bacterial counts in the re-circulatory systems showed a much more rapid reduction from the 3rd day (3.9 x 10^3 cfu/ml) itself and it reached a value of 0.4 x 10^3 cfu/ml on the 10th day.

Table 1. Total Plate Count in mussel meat (x 10^3 cfu/g)

Days	Control	Normal Seawater	Filtered Seawater	Re-circulatory system
1	37.38	29.13	33.95	32.20
3	43.69	15.58	19.29	12.91
5	50.00	14.39	14.44	3.55
7	43.41	14.04	3.38	0.81
10	42.58	12.96	2.48	0.68

Table 2. Total Plate Count in water (x10^3 cfu/ml)

Days	Control	Normal seawater	Filtered seawater	Re-circulatory system
1	25.58	26.23	17.49	16.90
3	28.54	30.43	15.92	3.90
5	31.50	21.07	13.30	0.70
7	28.03	29.45	5.42	0.53
10	24.55	18.85	4.63	0.40

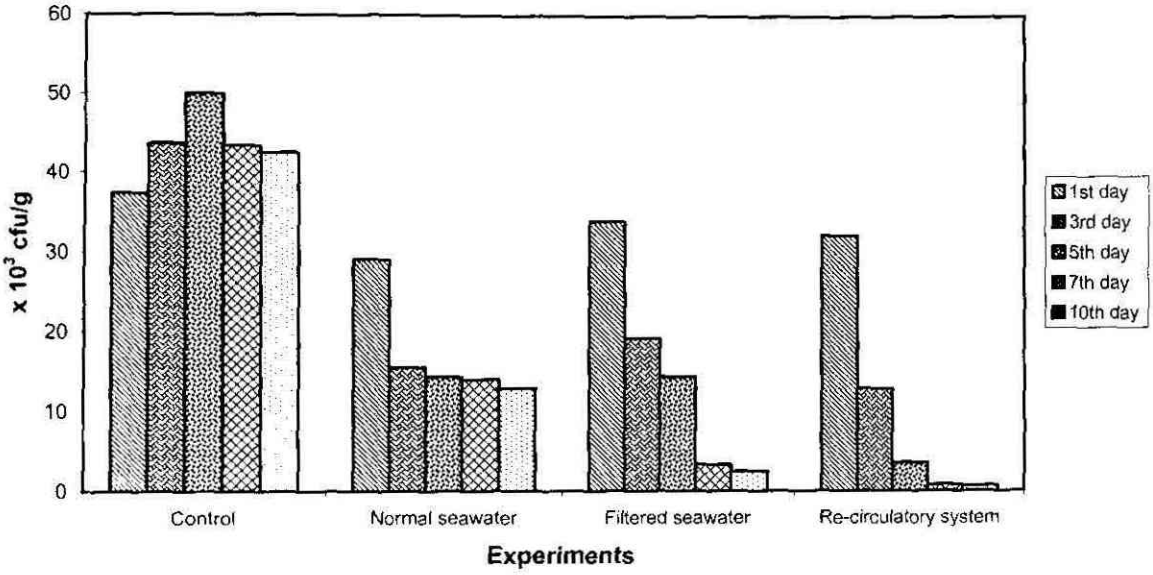

Fig. 5. Total Plate counts (TPC) in mussel meat

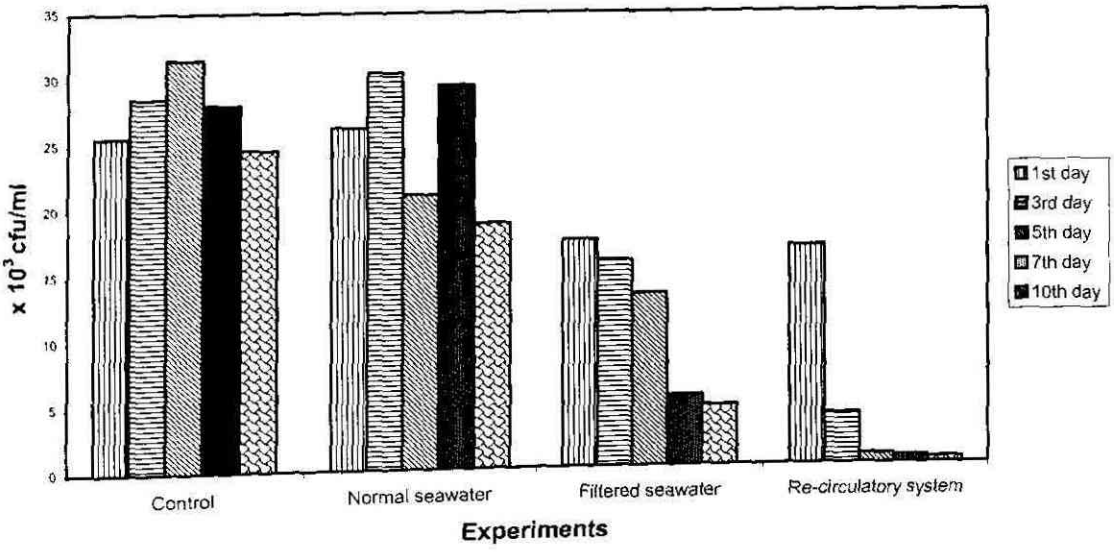

Fig. 6. Total Plate counts (TPC) in water

4.1.2 *Faecal streptococci* (FS) count

The faecal streptococci counts in the mussels were studied in the control and in the experiments, as it is another factor testing the bacterial quality of the mussels. The FS count in the 1st day samples of control was 2.63 x 10^2 cfu/g and it was more on the 5th day and again it was found reduced to 2.53 x 10^2 cfu/g on the 10th day (Table 3).

In the experiments with normal seawater the FS count was found similar to the control in the 1st day and there was a gradual decline in the subsequent days and it reached nil at the end of the experiment. The average FS count in the experiment with filtered seawater system on the 1st day was 1.15 x 10^2 cfu/g and it found decreased to 0.9 x 10^2/g on the 5th day and reached nil on the 7th day. There was a great decrease in the FS counts in the mussels in the re-circulatory systems. The faecal streptococci counts were found to be absent from 5th day onwards in this re-circulatory system. These observations revealed that the bacterial counts in the mussels could be reduced effectively through these systems with a minimum period and not affecting and/or improving the nutritional qualities of the mussels.

The average *faecal streptococci* counts in the water that hold the mussels were also studied and found a lower count in the water than the mussels. In the control the FS counts in the water on the 1st day was 0.92 x 10^2 cfu/ml and it was found increased in the following days and reached about 2.35 x 10^2 cfu / ml on the 5th day and then lowered and reached 1.57 x 10^2 cfu/ml at the end of the experiment (Table 4). The FS counts observed in the water of the experimental tanks with normal seawater on the 1st day was similar to the control. Observations on the 3rd day showed a sudden increase in the counts and it reached to 3.45 x 10^2 cfu/ml. However, there was a rapid reduction in the 5th day and it was found nil in the samples at end of the experiment on the 10th day. In the second sets of experiments with filtered seawater system also the FS counts increased slightly on the 3rd day but there was great reduction in the *faecal streptococci* counts in the subsequent days and reached nil on the 7th day. In the experiment with re-circulatory system there was no *faecal streptococcus* from the beginning of the experiment and it remained as such to the end.

Table 3. *Faecal streptococci* count in mussel meat ($x10^2$ cfu/g)

Days	Control	Normal seawater	Filtered seawater	Re-circulatory system
1	2.63	2.67	1.15	0.80
3	3.09	1.45	1.12	0.50
5	3.55	1.35	0.90	Nil
7	2.95	1.20	Nil	Nil
10	2.53	Nil	Nil	Nil

Table 4. *Faecal streptococci* count in water ($x10^2$ cfu/ml)

Days	Control	Normal seawater	Filtered seawater	Re-circulatory system
1	0.92	0.90	0.30	Nil
3	1.64	3.45	1.00	Nil
5	2.35	1.00	0.05	Nil
7	1.96	1.05	Nil	Nil
10	1.57	Nil	Nil	Nil

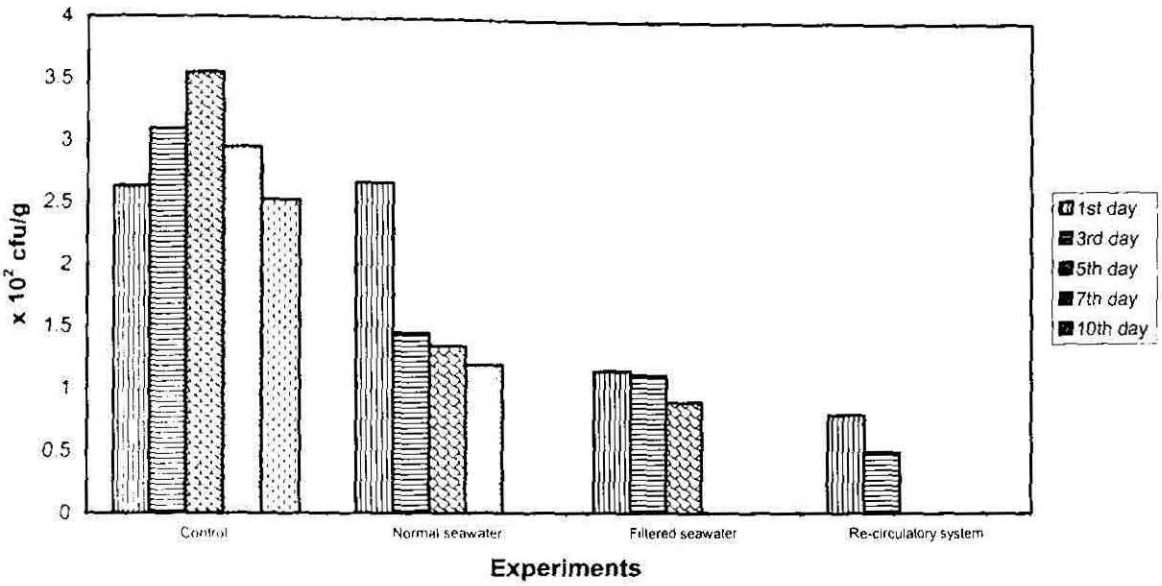

Fig. 7. *Faecal streptococci* count in mussel meat

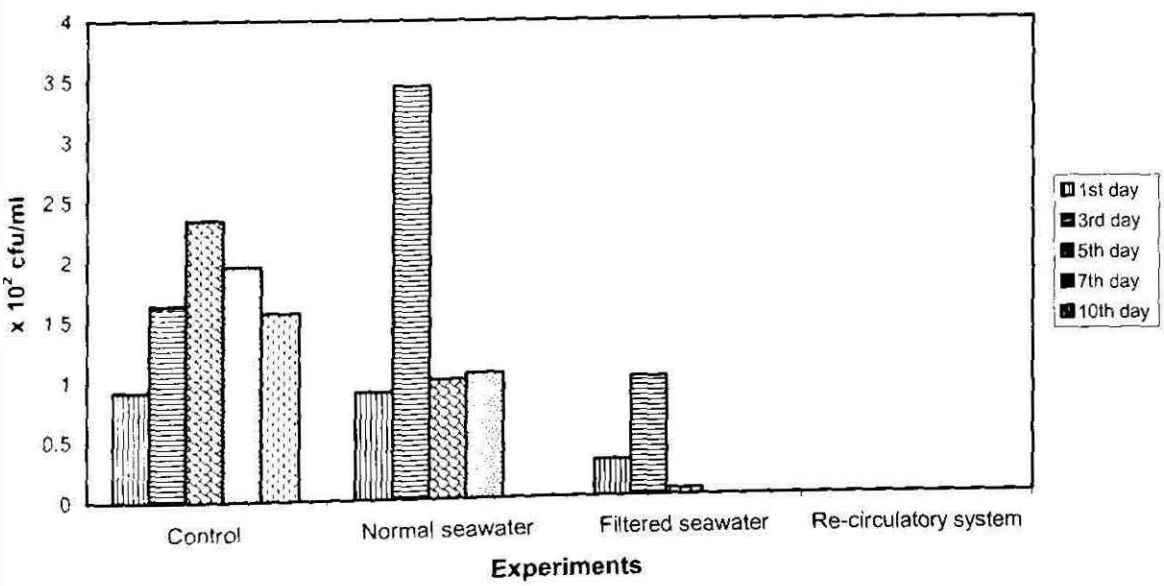

Fig. 8. *Faecal streptococci* count in water

4.1.3 Total coliforms (TC)

The presence or absence of coliforms in mussel is considered as a major factor of the bacterial quality and therefore the total coliform counts in mussel meat as well as in the holding water were also analyzed periodically in all the three sets of experiments along with the control and presented in table (5). It was found that, the total coliforms on the 1st day in mussel and surrounding water were 4.5 MPN/g and 8 MPN/100 ml respectively in control. In the 5th day samples of the control the total coliform counts remained same as that of the 1st day in mussels but the count was comparatively less in water. However, the total coliform count increased considerably on the 10th day in both the mussels and water to 9.5 MPN/g and 8 MPN/100 ml respectively.

The range of coliforms occurred in the mussels as well as water were 4.5 MPN /g and 8 MPN /100 ml respectively observed in the experiments with normal seawater on the 1st day. A sharp reduction observed in the counts in both mussel and water in the experiments with natural seawater in the following days. In the mussel it became 1.5 MPN /g and 1 MPN/100 ml in water on the 3rd day of the experiment. The coliform counts in the mussels reduced further to 0.7 MPN /g on the 5th day of the experiment and it found absent in the water on the same day. The coliform bacteria were completely absent in the mussel as well as in the water from 7th day to the end of the experiment.

In the experiments with filtered seawater, the coliforms counts were found 0.9 MPN /g in mussel and 1MPN /100 ml in water on the 1st day of the experiment. On the 3rd day it was found reduced to 0.4 MPN /g in mussel and absent in the water. From 5th day onwards the coliform bacteria were absent till the end of the experiments. Total coliform counts in the experiments with re-circulatory system were found 0.4 /g in mussel on the 1st day and it was absent in the water. The coliform bacteria were found completely absent from the 3rd day to the end of the experiment.

Table 5. Total coliforms in mussel meat (MPN /g) and water (MPN /100 ml)

Days	Control		Normal seawater		Filtered seawater		Re-circulatory system	
	Meat	Water	Meat	Water	Meat	Water	Meat	Water
1	4.5	8	4.5	8	0.9	1	0.4	Nil
3	4.5	8	1.5	1	0.4	Nil	Nil	Nil
5	4.5	4	0.7	Nil	Nil	Nil	Nil	Nil
7	4	4	0.4	Nil	Nil	Nil	Nil	Nil
10	9.5	8	Nil	Nil	Nil	Nil	Nil	Nil

4.1.4 Faecal coliforms (FC)

Presence of faecal coliforms was studied in both the mussel and water samples of all the experiments as well as in the control. Faecal coliforms were found in less numbers in the mussels as well as in the water that held these mussels in the control and in all the three sets of experiments. The faecal coliform counts in the mussels were 2.5/g on the 1st day (Table 6). The present studies revealed that the FC counts in the mussel reduced to 1.5/g on 5th day. Whereas on the 10th day of the study it was found increased again and reached 2.5/g in the control.

The surrounding water showed a faecal coliform count of 1/100 ml from the 1st day to the 7th day and it increased to a higher value of 3/ 100 ml on the 10th day. In all the experiments it was found that faecal coliforms were completely eliminated from the mussels as well as from the water from 3rd day onwards. The faecal coliform counts in the mussels were 1.5/g in the experiments with normal seawater and filtered seawater, and it was 1/g in the experiments with re-circulatory systems during the early days of the experiments. The faecal coliform count was 1.5 /100 ml in the water in the experiments with normal seawater and in the other two experiments it was 1 /100 ml. From the studies it is found that the faecal coliforms could be eliminated through these systems and thus the quality of the mussels could be improved before marketing.

Table 6. Faecal coliforms in mussel meat (MPN /g) and water (MPN / 100 ml)

Days	Control		Normal seawater		Filtered seawater		Re-circulatory system	
	Meat	Water	Meat	Water	Meat	Water	Meat	Water
1	2.5	1	1.5	1.5	1.5	1	1	1
3	2.5	1	Nil	Nil	Nil	Nil	Nil	Nil
5	1.5	1	Nil	Nil	Nil	Nil	Nil	Nil
7	1.5	1	Nil	Nil	Nil	Nil	Nil	Nil
10	2.5	3	Nil	Nil	Nil	Nil	Nil	Nil

4.2 Condition index of animals

From the investigations it was found that average condition index of the animals used in the experiments varied from 127 to 132.8 (Fig. 9). The maturity stages of the animals used in the experiments varied between immature to spent. The average CI of the mussels used in the first 2 experiments with normal seawater system and filtered seawater system was 132 while that of the control was 130. In all the above cases, the gonads were white coloured with immature oocytes. Where as, in the third experiment using re-circulatory seawater system was 127 and their ovaries were flaccid and loose indicating the spent conditions. The changes in the condition index of the mussels were observed on the 3rd, 5th 7th and 10th days in all the set of experiments. The observations revealed that the CI of the mussels increased gradually in experiments with normal sea water system and filtered seawater system. Even though there was an increase in condition index of control mussels, this increase was more prominent in the experimental mussels when compared to control.

In normal seawater system there was a gradual increase from 1st day (132) to the end of the experiment (138) even though there was a slight decrease on the 7th day. In filtered seawater system a steady increase was observed from 1st day to the 10th day and 143 was attained as maximum. Where as, in the re-circulatory system the condition index was found to be suddenly decreased to 114 in the 3rd day from an initial of 127. After the third day it was gradually increased to the termination of the experiment and reached a final value of 130 on the 10th day.

Condition index of the mussels used in the re-circulatory system was low as these animals were in spent conditions. However, the CI of animals was found increasing in the experimental period as the gonadal conditions are progressing. The present investigations revealed that the CI could be modified with a short duration provided good feeding and water quality before marketing. Statistical analysis using Analysis of Variance technique indicated significant difference between control and all set of experiments ($P< 0.05$).

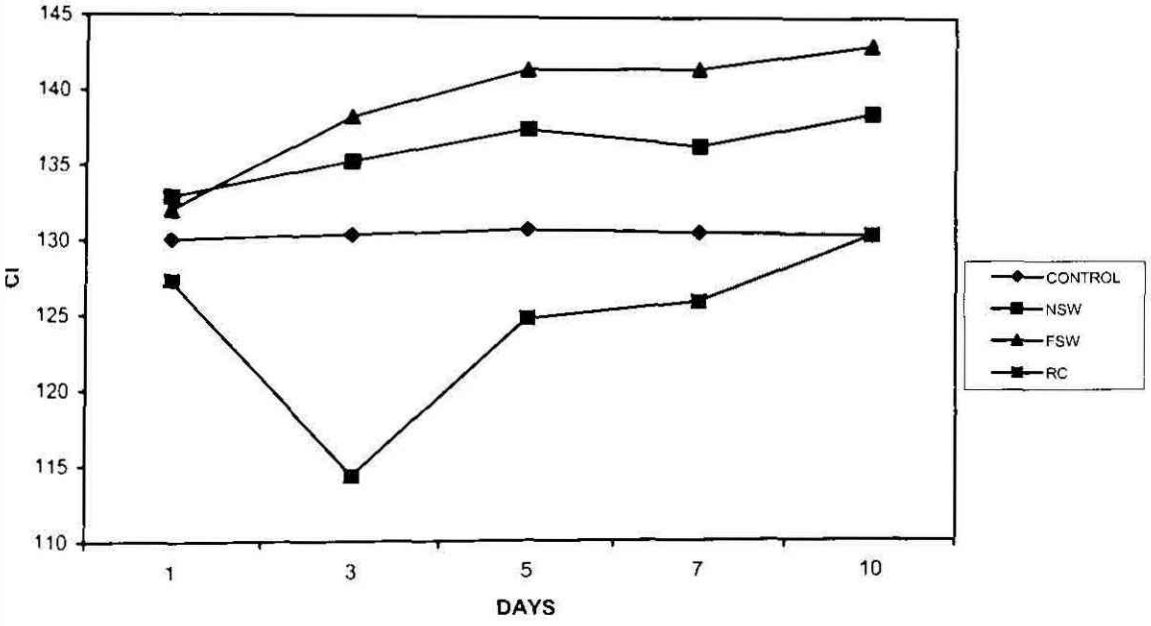

Fig.9 Condition index of animals

4.3 BIOCHEMICAL COMPOSITION OF MUSSEL MEAT

4.3.1 Moisture content

The average moisture content of the mussels in the control as well as experiments with normal seawater systems and filtered seawater systems was 78%; where as the average moisture content of the mussels used in experiments with re-circulatory system was 80 % (Fig. 10). Analysis of variance revealed that experiments with re-circulatory system showed higher value than the others ($P< 0.05$).

A gradual decrease in the moisture content was noticed in all the experiments and in the control during the experimental period of 10 days. The moisture content of the mussels in the experiments with normal seawater system and the filtered seawater system decreased steadily and reached 76 % and 75 % respectively with the 10 days. The moisture content was found decreased with the progression of the reproductive condition of the gonads in these experiments.

In the experiments with re-circulatory system a sudden increase in the moisture content was noticed on the 3rd day and it attained 84% which can be attributed to the post spawned condition of the mussels used in the experiment. After that, from 5th day onwards a gradual decrease was observed till the end of the experiments as the gonadal stages are progressing. Even though the percentage decrease was found highest in the filtered seawater system a significant difference was observed in the other two sets of experiments also when it was compared with the control.

4.3.2 Dry meat weight

The present study revealed that the percentage of dry meat weight in the experimental mussels increased with the 10 days of the experiment. The percentage of dry meat weight was found inversely proportional to the moisture content. The average dry meat weight was found 21% in control and there was

gradual increase in it in the subsequent days (Fig. 11). In the experiments with normal seawater an overall increase in the dry meat weight was observed even though a slight decrease was observed on the 7th day.

There was reasonable increase in the dry meat weight of the mussels in the filtered seawater system. The average percentage of the dry meat weight of the animals in the beginning of the experiments was 19% in the re-circulatory system and a rapid decline to 15% on 3rd day. However, from 5th day onwards, it gradually increased and reached a final value of 19% on the 10th day. The analysis of variance indicated significant difference between all set of experiments ($P< 0.05$) than the control. The dry meat weight was found to increase as the reproductive stages of the animals progressed.

4.3.3 Ash content

The average percentage of ash in the initial samples of control was 8.2% and a slight decrease was found during the first half of the experimental period (Fig. 12). It increased in the subsequent days and reached 12% as maximum at the termination of the experiments. Similarly a decreasing trend was observed in the average ash content up to 5th day in the experiments with normal seawater systems but it slightly increased in the 7th day and again a slight decrease was found on the 10th day. Statistically, experiments with normal seawater system were found to be superior to others ($P< 0.05$).

An increasing trend of ash contents was noticed in the experiments with filtered seawater system throughout the period of experiment. The ash content in the initial samples was 6.6% and reached the maximum of 9% as maximum on the 10th day at the end of the experiment. Where as in the experiments with re-circulatory seawater systems the percentage of the ash content remained almost steady throughout the experimental period and a sudden increase from 8% to 11% was observed on the 10th day.

4.3.4 Protein

The average protein percentage in the mussel meat was 62% in the initial samples of control (Fig. 13). A gradual decline in the protein content was observed in the control samples in the following days of the experiment, where as a significant increasing trend in percentage protein content was observed in the experiments with normal seawater and filtered seawater systems. Analysis of variance indicated insignificant difference between experiments with normal seawater and filtered seawater systems ($P > 0.05$). The percentage protein content in the experiments with normal seawater system increased from 65% to 73% and in the experiments with filtered seawater system it increased from 62% to 75% in the duration of 10 days.

Even though a sharp decline in the protein content of the mussels were noticed in the experiments with re-circulatory system during the initial days (i.e. from 56% to 52 %) however, from the 3rd day onwards the percentage protein content showed an increasing trend till the end of the experiment indicating the progress of the reproductive stages of animals.

4.3.5 Carbohydrate

The average carbohydrate content in the control animals was 14.2% in the 1st day (Fig. 14). The present investigations revealed that the carbohydrate contents in the control animals kept in the natural environment increased; the increase was very slow till the end of the experiment. The percentage of carbohydrate in the mussels used in the control increased from 14 % to 15.5% within 10 days. Where as in the experiments with normal seawater system, the carbohydrate percentage increased from 14% to 18% with a short period of 10 days.

This increase in the percentage of carbohydrate content in the mussels was more significant in the experiments with filtered seawater systems. The initial carbohydrate content was 16.9 % and it reached 23% in the termination of the experiment on the 10th day. A total of around 7% increase was observed in the

carbohydrate contents of the experimental animals with the 10 days. The average carbohydrate contents of mussels used for the experiments in the re-circulatory system was around 13% on the 1st day and there was a rapid decline in the percentage on the 3rd day and it reached 9%. After that it was found to increase gradually till the end of the experiment and reached 13.8% as its ovarian conditions are progressing. By the Analysis of Variance it was found that there was significant difference in all set of experiments ($P < 0.05$).

4.3.6 Total Lipids

From the present investigations it was found that there was not much marked difference in the lipid contents in the control or experimental mussels in these short-term treatments. Experiments with normal seawater showed almost constant value for the lipid contents up to 7th day but a slight decrease was observed at the end of the experiments (Fig. 15). The percentage lipid was 11% in the beginning and remained constant till the 7th day but a slight decrease observed on the 10th day samples and it reached 10.45%.

In the experiments with filtered seawater system the lipid percentage was 10% up to 3rd day; a sudden increase was noticed in the 5th day and it reached 12%. However, a gradual decrease found in the subsequent days of the experiment and finally reached 11.5%. Altogether a slight increase in the lipid content was recorded in the experiments where filtered seawater systems were used. Similarly a slight but increasing trend of lipid content was observed in the experiments with re-circulatory systems. The lipid content of the mussels in these experiments was found to increase from 9% to 10% even though there was slight decrease in the 3rd day. Analysis of Variance technique showed significant difference between control and all the other set of experiments ($P<0.05$).

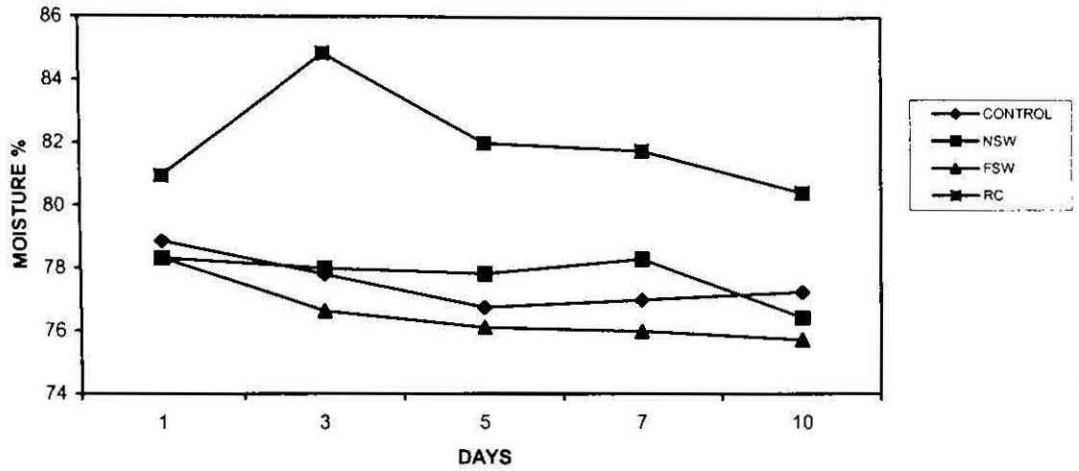

Fig. 10 Moisture content

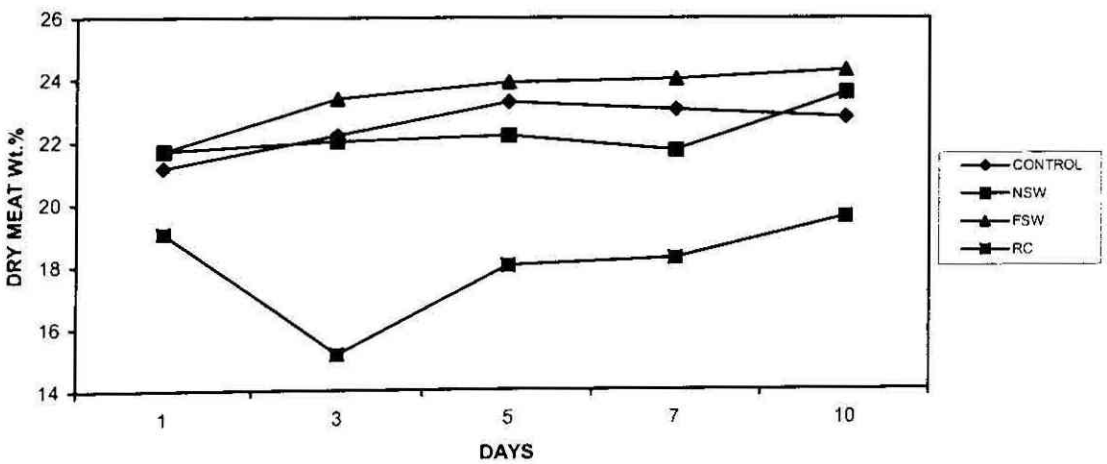

Fig. 11 Dry meat weight

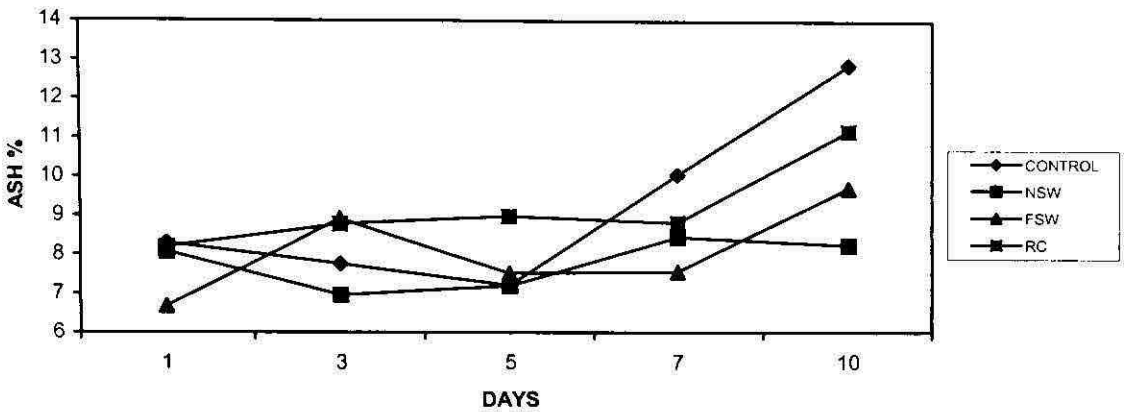

Fig. 12 Ash content

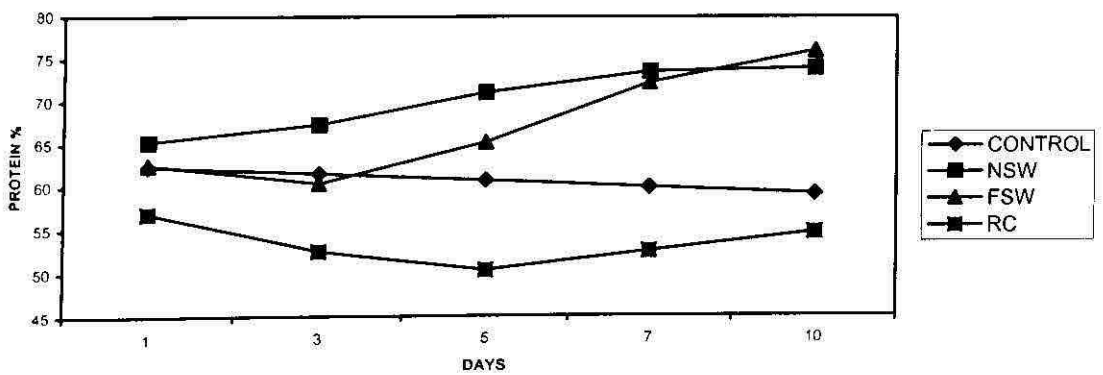

Fig. 13 Protein

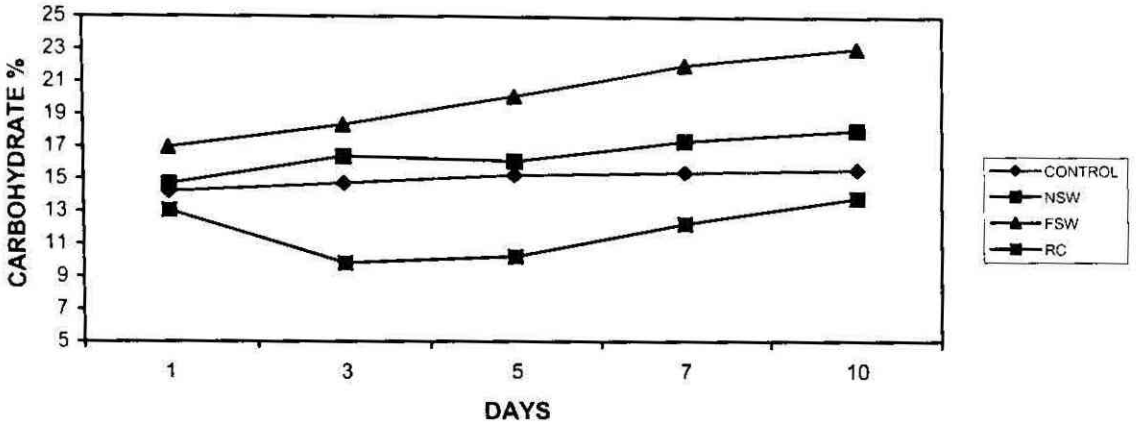

Fig. 14 Carbohydrate

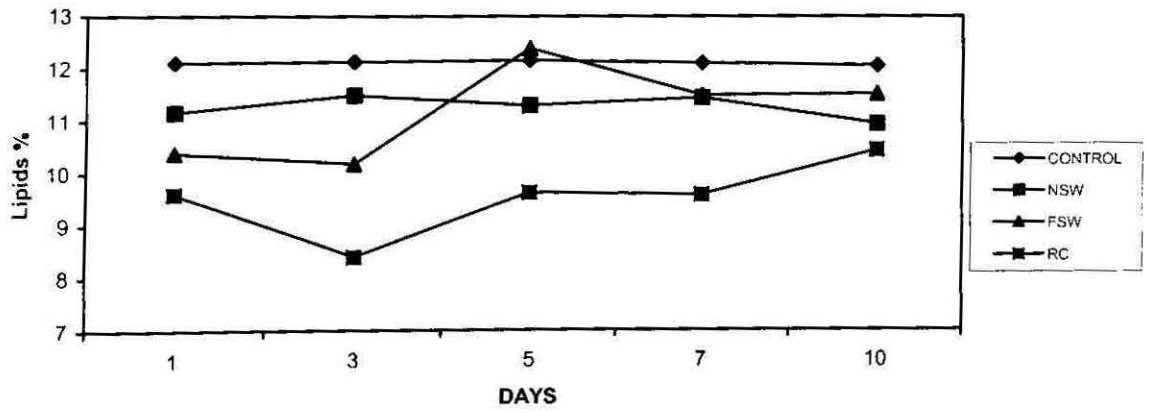

Fig. 15 Total Lipids

Discussion

5. DISCUSSION

The sessile habit of mussels' makes, in fact, their biochemical composition strictly dependent on the phytoplankton resources available (Orban *et al.*, 2002; Perez Camacho *et al.*, 1995) and the biochemical composition of mussels varies with the feeding, reproductive activity, and physiological condition and also due to external stresses like starvation and desiccation. Food availability has been regarded as one of the most important factors influencing the growth of bivalves (Danovaro and Fabiano, 1997; Mandon *et al.*, 1998; Sara *et al.*, 1998). The filter feeding habit of the mussels accumulate microorganisms and water borne contaminant through lower food chain organisms in their meat (Viarengo and Canesi, 1991; Widdows and Donkin, 1992; Dore *et al.*, 2003). The quality requisites of bivalve molluscs entrust both less bacterial population and at the same time high meat contents with a better proximate composition (Beninger *et al.*, 1984; Orban *et al.*, 2002).

Mussel meat is delicious, tender, easily digestible and at the same time cheapest and most nutritious shellfish meat in the world market. However, many researchers reported that being filter feeder and sedentary, mussels are more vulnerable to bacteriological and chemical pollution than other animals and transmit many diseases to man when consumed and are therefore, an ideal organism for monitoring environmental pollution (Aurora *et al.*, 1983; Richards, 1998; Rippey, 1994; Lees, 2000; Cliver, 1997; Dore *et al.*, 2003). Contamination of mussels, as a food commodity, poses a danger to public health (Hackney *et al.*, 1992 and Shumway, 1992).

The present study revealed that the mussels collected for the experiments harboured a large number of bacteria in their body from the surrounding water. The bacterial profile of the mussel meat and the water that held the mussels were similar and indicated the bacterial flora of mussels are coming from the surrounding water. The total plate count of the mussels used in the experiments as

well the control and the water samples from where these mussels are collected showed higher values than the permitted levels.

Investigations revealed that due to their filter feeding activities, mussels concentrated these bacteria in their body from the surrounding water. The major groups of bacteria found usually are *coliforms*, *E. coli, faecal streptococci* and occasional pathogens like *Salmonella, Shigella, Vibrio parahaemoliticus* and *V. choleracae*. Generally the profile of the bio-accumulated bacteria will be a true reflection of the bacterial profile of their environmental water. The total bacterial count of the surrounding seawater was consistently lower than the corresponding counts of the mussels. This is in agreement with the finding of Durairaj *et al.* (1983). Dato-cajegas and Lin, (1996) also reported that TPC usually present in greater number in mussels than in water at the same time due to the accumulation the bacterial in the meat while filtering the water.

Presence of *faecal streptococci* has been considered an excellent indicator of human and animal faecal pollution (Holdeman *et al.*, 1976). Present studies showed the presence of FS in the mussels collected for the experiments in higher counts than the surrounding water, which also contained FS. Balachandran and Surendran (1984) studied the distribution of faecal indicator bacteria in clams, mussels and oysters and in their aquatic environments and found that these bivalves harboured large bacterial populations including faecal coliforms, *Escherichia coli* and faecal streptococci. Based on these studies on the bacterial profile Surendran *et al.* (1986) concluded that mussel is a good indicator of faecal pollution of aquatic environment.

The FS counts remained in the control mussels in varying counts even at the end of the experiments however; the FS counts in the mussels were found completely eliminated in all the experiments at the termination of the experiments in the present studies. The elimination rate was highest in the re-circulatory systems (3 days) followed by filtered seawater (5 days) and normal seawater (7 days). Where as, Cohen and Shuval, (1972) and Anson and Ware, (1974) had reported higher survival of FS with respect to other bacteriological indicators in the marine environment. Aurora *et al.*, (1983) studied depuration of mussels by two different

methods and reported that they got an almost similar reduction levels for the FS in both the systems but very high numbers of these bacteria were present in mussels before and after depuration. However, in the present investigations the FS were found completely eliminated in all the experiments at the end of the experimental period.

Among the coliforms in human source 96.4% are faecal coliform and among animal source 93-98% is faecal coliforms (Geldreich, 1978). Faecal coliform bacteria are more directly associated with faecal contamination from warm-blooded animals than total coliforms, and FDA has approved their use as an indicator of faecal pollution in market level shellfish (USFDA, 1984). The present investigations revealed that total coliforms and *E. coli* were present in the mussels collected for the experiments as well as in the control mussels. Total coliforms and *E. coli* counts were found to increased counts in the control mussels and in the surrounding water at the end of the experiments. While in the experiments the counts were found decreased and finally eliminated completely in all the sets. In the experiments with normal seawater the complete elimination of total coliforms were found on the 10^{th} day. Where as only 3 days were taken in the filtered seawater systems and only one day in the re-circulatory systems for the complete elimination. Thi Son and Fleet (1980) reported depuration reduction of coliforms and *E. coli* up to 97 % and Aurora *et al.*, (1983) reported 90 % reduction in the coliform bacterial counts through depuration of mussels. Present studies revealed that during captivity, the bacterial load was considerably reduced as the water used is devoid of the contaminants. TPC and TC in the depuration water were increased due to the multiplication of bacteria and / or re-suspension of discharged faeces from the tank (Delvin and Eng, 1973).

Generally purification of bacteria contaminated bivalves has been effected by depuration, a technique first developed at the Fisheries Experimental Station, Conway, U. K. by Dodgson, (1928), utilizing their own physiological filtration mechanism. Afterwards the technology of depuration has well studied by many researchers like Huntely and Hammerston 1971; Furfari, 1976; Neilson *et al.*, 1978; Souness *et al.*, 1979; Nambudiri *et al.*, 1995; Heath and Pyke 2002; Dore *et al.*,

2003 according to their conditions. Most countries have chosen to clean their shellfish in depuration plants rather than by relaying in natural waterways.

Ultraviolet irradiation, ozonation, and chlorination etc are widely used to sterilize seawater for depuration (Kelly 1961; Wood, 1961; Souness et al., 1979; Richards, 1998). The bacterial load of the mussels could be brought down effectively either by keeping in filtered seawater or by keeping in re-circulatory system (quality could be further improved). Heath and Pyke (2001) developed a scallop depuration system using natural seawater only. In all these studies it is found that the depuration relies on bivalves continuing filter-feeding activity when placed in tanks of clean seawater and purging themselves on contamination. In some of the depuration systems the animals are found to remain closed due to the presence of new components like chlorine in chlorinated seawater.

Usually in the depuration systems the animals are subjected to starvation and therefore the time that keeps the animal in this system is a main limiting factor (Huntley and Hammerston, 1971; Souness *et al.*, 1979) and it affects the nutritional quality adversely. Where as if, the animals are keeping in good quality seawater with feeding the time is not a critical factor and it did not deteriorate the meat quality; instead it improves the same to some extent. More over the presence of feed in the seawater can induce the filter-feeding activity of the animals much earlier than in a system without feed. Nambudiri *et al.* (1995) stated that increased filtration rates could eliminate the bacteria more rapidly.

Biochemical composition: Mussels possess a very high position from the nutritional points of view, as they are high in protein and iron and are very low in calories, fat and cholesterol. Stress conditions, environmental situations requiring major energy expenditure or gametes release, may be responsible for the poor meat content and biochemical composition (Orban *et al.*, 2002). The differences in biochemical composition are large enough and to be considerable important to shellfish farmers, harvests and processors. The magnitude of weight and biochemical changes in flesh content may also be significant for consumers (Ansari *et al.*, 1981). Studies have demonstrated that the biochemical composition of bivalves clearly reflects the environmental conditions and food availability (De

Moreno *et al.*, 1976; Napolitano *et al.*, 1992; Fernandez Reiriz; *et al.*, 1996; Soudant *et al.*, 1996). The biochemical composition of the mussels varied predictably, with loss of water and accumulation of storage or reserve materials (Okumus and Stirling 1998).

Condition index: Condition index has been used as a measure of quality and health of harvested mussel (Okumus and Stirling, 1998). In the present investigation it is found that the condition indices of the animals used in the control, and the first 2 experiments were higher compared to the mussels of the 3rd experiments as the animals use for these experiments were at the spent stage. Numerous workers have demonstrated that the condition index of the various species of bivalves from boreal and temperate coastal varies seasonally and is mainly related to the level of available food and the annual reproductive cycle (Baird, 1958, 1966; Westley 1970; Walne, 1970; Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Gabbott and Stephenson, 1974; Dare, 1976). In addition to these in a detailed field study, Walne (1970) has studied that meat weight, condition index and all biochemical compositions of bivalve declined when the food levels are low. The same opinion has been reported by several investigators like, Bayne and Thompson, 1970; Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Gabbott and Stephenson, 1974.

In the present study the condition index and the dry meat weight of animals maintained in natural seawater and filtered seawater was found increased tremendously compared to the control. This was due to the availability of sufficient feed in the experimental condition. Even though, the condition index, dry meat weight and all biochemical composition of animals kept in re-circulatory system were less compared to the other two sets of experiments as these animals were at the spent condition. However the condition index of these animals was found to significantly increase during the experimental period. Changes in condition indices resulted from the complex interaction of a variety of factors including food, temperature and salinity, but food supply is the most important factor after the gametogenic cycle (Hickman and Illing worth, 1980; Small and Van Stralen, 1990). A close relationship has also been reported between the gametogenic cycle, condition index and the storage-consumption cycle of reserves and meat quality

(Gabbott and Bayne, 1973; Dare and Edwards, 1975; Gabbott, 1975). Fairly good agreement has been found between the patterns of biochemical composition, the condition index and gametogenic cycle described by Seed, (1975, 1976). Orban *et al.*, (2002) described a decrease in the condition index coincided with the spawning period. Condition index, measures the plumpness of meat. The criterion classifies CI > 120 as plump, 100 – 119 as excellent, 80 –99 as good and < 79 as fair.

Moisture content: Fluctuations in the moisture content due to absorption of water and loss of solids from the body of animals are the most significant feature of the changes in the chemical composition of the meat. These changes in meat during certain periods lower their commercial value also (Thangavelu and Sanjeevaraj, 1988). In all experiments and control it was found, there was a reciprocal relationship between the water content of the body and the biochemical constituents. This agrees with the view of Durve and Bal (1961) in *Crassostrea gryphoides*, Galtsoff (1964) in *C. virginica*, Nagabhushanam and Mane (1975) in *Katelysia opima* and Nagabhushanam and Talikhedkar (1997) in *Donax cuneatus*. Joshi and Bal, 1965; Deshmukh, 1972 have reported a similar relationship in other bivalves.

A very high moisture content observed in mussels in the re-circulatory system compared to the control and other 2 sets of experiments which can be attributed to the spent condition of the animals. However, in all sets of experiments including the spent mussels the moisture content was found decreased as the gonadal conditions are progressing due to the availability of food. The decrease in moisture content was very less is in the control. Ansell *et al.*, 1973 stated water content of the bivalve might give an indication of the time of spawning.

Dry meat weight: Changes in the body weight, body component index, percentage of water in the body in relation to environmental and physiological aspects of bivalves has been studied by several workers (Venkataraman and Chari, 1951; Durve and Bal, 1961; Durve 1964; Joshi and Bal 1965; Ansell et al; 1973; Nagabhushanam and Mane, 1975; Nagabhushanam and Talikhedkar, 1975; Nagabhushanam and Dheshmukh, 1997; Rivonker et al., 1995; Stirling and Okumus,

1995) and opined that the body weight depending upon the intake of food, reproductive activity and changes in the metabolic activity of the animal. In the present investigation maximum dry meat weight of 24.2% was obtained in the in the mussels on 10th day of the experiment with filtered seawater system where the gonadal conditions were also found progressed maximum. Durve, (1964) and Ansell *et al.*, (1980) reported that the meat weight varies depending upon the intake of food, reproductive activity and changes in the metabolic activity of the animal. Body weight of the mussel was at minimal soon after extrusion of gametes from the animal and increases gradually towards the onset of gametogenesis (Thangavelu and Sanjeevaraj, 1988).

The decline in flesh weight during spawning season was mainly due to the change in gonadal phase (Ansari, 1981). Loss of flesh weight has been attributed to food scarcity and spawning. Maximum water percentage of the soft tissue coincided with minimum flesh weight was found in the mussels used for the 3rd of experiments during the present studies. The increase in dry meat weight was minimum in the control than the experiments, which may be due to the reduction of food in the natural environment compared to the experimental tanks. It is known that water temperature; food availability and reproductive cycle of animals may influence the meat yield and biochemical composition of mussels (Fernandez Reiriz *et al.*, 1996; Okumus and Stirling, 1998).

Ash content: The maximum ash contents of 12% observed on the 10th day in the control but the same did not show a definite pattern in the experiments though an over all steady increase occurred in the experimental mussels. Similarly, Rivonker and Parulekar, (1995) estimated the ash contents of the mussels grown on the raft but could not find any definite patterns with other factors. Biochemical composition of the bivalve molluscs, *Villorita cyprinoids* and *Meretrix casta* were done in relation to season and species and concluded that the ash content increased may possibly due to an increased inorganic content in the body constituents (Lakshman and Nambisan, 1980). Here in the present investigation also the highest ash content observed in the control is attributed to the high inorganic contents, as it is found not correlated to any factors studied.

Protein: Mussel meat is a protein rich food. Protein, a source of energy reserve in bivalves and it plays an important role as compared to glycogen and other intermediary carbohydrate metabolism (Rivonker and Parulekar, 1995). Higher levels of protein were reported just prior to the spawning period in the green mussel *P. viridis* because of the increased food availability (Rivonker and Parulekar, 1995). The present investigation also revealed that the protein levels in the mussels were high in the first 2 experiments because of the increased food availability and the resulting reproductive progress in the animals. Even though the initial protein levels are almost similar in the control and the in the first two experiments the protein levels were found significantly increased in the experimental mussels. Decrease of protein content in the control might be due to the less availability of food in the nature compared to the experimental tanks. Nagabhushanam and Mane, (1978) and Wafer *et al.* (1976), opined that the increased protein content in the bivalves in the pre-spawning conditions might be a mechanism of storage of reserves to meet spawning requirements. Another possible reason for elevated protein content could be the increased feeding efficiency associated with food availability thereby resulting in proper assimilation of food and better metabolic conditions at that time as stated by (Quasim *et al.*, 1977).

Whereas in the experiments with re-circulatory system, feed was available as in the other two experiments but the animals were at spent conditions and due to the same reason the protein content found decreased in the initial stages; but a gradual increase occurred in the later stages due to the higher food availability. Rivonker and Parulekar (1995) reported an increase in protein content during pre-monsoon season in coincidence with the maturation of gonads. Similar observations have also been reported earlier (Nagabhushanam and Mane, 1978; Wafer *et al.*, 1976). Ansari et al., 1981 again reported higher levels of protein in bivalves in all seasons except breeding time. A seasonal cycle characterized by phases of storage and depletion of reserves, reflecting the stage of gonadal development as well as the availability of food have been reported (Fernandez-Reiriz, *et al.*, 1996; Okumus and Stirling, 1998; Orban *et al.*, 2002).

Carbohydrate: Mussels contains substantial quantities of glycogen, which is primarily responsible for its characteristic sweet flavor. The carbohydrate of bivalves comprised mainly glycogen (Gabbott and Bayne, 1973) and the changes in carbohydrate may be due to accumulation and utilization of glycogen at different stages like gametogenesis and spawning (Ansari *et al.*, 1981). Gabbott and Bayne, (1973) reported, a distinct sequence of metabolic events during reproduction in which a period of storage metabolism, marked by an increase in percentage carbohydrate content, is usually followed by a period of gametogenesis, marked by carbohydrate depletion. In the present investigations the percentage carbohydrate was found increased significantly in the first two experiments; however the increase was very less and slow in the control. Whereas in the third set of experiments with re-circulatory system the carbohydrate content found decreased in the initial phases though plenty of food was available in the experimental systems may due to the spent conditions of the animals. Accumulation and depletion of the stored reserves in bivalves depends on the stage of gonadal development, environmental influences on metabolic activities and the quantity and quality of available food (Gabbott and Stephenson, 1974; Pieters *et al.*, 1979).

The maximum carbohydrate content (23 %) observed in the experiments with filtered seawater in the present studies could be attributed to the progress of the reproductive stages and increased feed availability with minimal energy expenditure for other environmental stress conditions. Carbohydrate content rose to the maximum due to high intensity of feeding and thereby the weight of the oyster also increased considerably. (Thangavelu and Sanjeevaraj, 1988). Ansell *et al.*, 1973 have reported that in bivalves generally the carbohydrate reserves may be rapidly utilized under unfavorable condition and the great variations in the tissue indicates that the level of mobilizable carbohydrate reserves may fluctuate widely and rapidly in response to fluctuations in condition affecting the nutrition of the animal. A close relationship has also been reported between gametogenic cycle, condition index and storage consumption cycle of reserves, particularly glycogen and meat quality (Gabbott, 1975). Okumus and Stirling (1998) reported an accumulation of glycogen and decline in lipid coincidence with low meat yields and condition index values with spawning was by in mussels cultured in two Scottish sea lochs.

Total lipids: Lipid content of the meat remained almost steady throughout the period of study with a little increase at the end of the experiments in filtered seawater and re-circulatory systems in the present investigations. The increase in the lipid content might be due to the increased feeding in the experimental tanks. Higher values of lipid in the initial stages were mainly due to increased feeding efficiency and fairly steady lipid contents with little fall in the values during the spawning season has been reported in relation to seasons in mussels by Chu *et al.*, (1990). Venkataraman and Chari (1951) correlated high lipid level with intensive feeding and storage of fat and low values on later stages before spawning in oysters. Low values were mainly due to utilization of accumulated lipid for building up of tissue material. Another probable cause for low lipid values could also be due to initiation of gametogenesis and utilization of energy reserves for development of gametes. Quasim *et al.*, (1977), Rivonker and Parulekar, (1995) reported that at this time water content was high indicating an inverse relation between lipid and water.

Summary

SUMMARY

The salient points of the studies on the impacts of short-term environmental manipulations in the bacterial quality and on the nutritional quality of the mussel *Perna viridis* collected from an area of commercial fishery are given below:

1. The bacterial contaminations of the mussels collected from the commercial fishery sites and the surrounding water were examined for total plate count, faecal streptococci count, total coliforms and faecal coliforms and the studies revealed that the mussels and the surrounding water harboured many bacteria including faecal contaminants and the bacterial load was high in mussel meat than the surrounding water.

2. The total bacterial count (TPC) of the samples varied from 37 x 10^3 cfu/g to 50 x 10^3 cfu/g in mussel meat and from 25.58 x 10^3 cfu/ml to 31.5 x 10^3 cfu/ml in the natural seawater during the experimental periods and the experiments reduced the total bacterial count of mussel meat to 12. 96 x 10^3 cfu/g, 2.48 x 10^3 cfu/g and 0.68 x 10^3 cfu/g and the surrounding water to 18.85 x 10^3 cfu/ml, 4.63 x 10^3 cfu/ml and 0.40 x 10^3 cfu/ml respectively in the normal, filtered and re-circulatory seawater systems.

3. The faecal streptococci count in the samples varied from 2.63 x 10^2 cfu / g to 3.55 x 10^2 cfu / g in mussel meat and from 0.92 x 10^2 cfu / ml to 2.35 x 10^2 cfu/ml in natural seawater during the experimental tenure and the experiments could completely eliminate the faecal streptococci count of the mussel meat with 10, 7 and 5 days and the surrounding water with 10, 7 and 1 days respectively in normal, filtered and re-circulatory seawater systems.

4. The total coliforms of the samples varied from 4.5 MPN/g to 9.5 MPN/g in the mussel meat and from 4 MPN/100 ml to 8 MPN/100 ml in the natural seawater during the experimental tenure. The complete elimination of the same in the mussel meat could be effected on 10^{th}, 5^{th}, 3^{rd} days and in the water on 5^{th}, 3^{rd},

and 1st days of the experiments in the normal, filtered and re-circulatory seawater systems respectively.

5. The faecal coliforms of the samples on the day of collection varied from 1.5 MPN/g to 2.5 x 10^3 MPN/ g in mussel meat and from 1 MPN/100 ml to 3 MPN/100 ml in the natural seawater during the experimental tenure. The complete elimination of the same in the mussel meat and the surrounding water could be effected on 3rd day in the normal seawater and with 1 day in other two (filtered and re-circulatory) systems.

6. The condition index (CI) and biochemical composition of the mussel meat assessed just after its collection to assess the changes in these factors within the 10 days of the experiments with feeding. Significant increase occurred in condition index (130- 143), dry meat weight (21% -24%), protein (62% -75%), carbohydrate (14% -23%) compared to the initial value as well as to the increase observed in the control with the same period.

7. The moisture content decreased while the ash contents and the lipids remained almost constant throughout the period though a slight increase was there in the filtered seawater and re-circulatory systems at the end of the experiments.

8. Present investigations revealed that among the three treatment studies, re-circulatory system was found as the most effective for eliminating the bacteria with minimum time followed by filtered and normal seawater systems.

9. Highest increase in biochemical composition occurred in the filtered seawater followed by the normal seawater systems. The increase in the re-circulatory system was comparatively less and it was due to the spent conditions of the animals used in that system.

10. The investigations revealed that the total quality of the mussels in the commercial catches could be increased in terms of the bacterial quality and the nutritional aspects through a short term make up touch to attract the consumers

and the markets that will increase the export demand as well as value and it can ultimately promote the mussel culture of the country.

CONCLUSIONS ...

Points for improvement of mussels' mariculture

- The present study revealed that the bacterial contamination of the mussels coming from the surrounding environment through filter feeding could be eliminated in short periods by keeping the harvested mussels in captivity using quality seawater and quality feed.

- Adequate feed supplements during the periods of rearing in captivity can improve the meat quality in terms of nutritional factors like condition index, carbohydrate, proteins etc. to some extent as a process of value addition just prior to marketing of the product for attracting consumers.

- Larger ponds with quality seawater and addition of phytoplankton cultures can be used in the fields for fattening the harvested products in case of mussel culture or natural fishery.

- The products thus improved the qualities can be branded as quality mussels or value added mussels and marketed at higher prices with a label of its bacterial load and nutritional details

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